Improvement on cellulose accessibility and reactivity of different wood pulps

Viviana Köpcke

KTH Chemical Science and Engineering

Licentiate Thesis

Royal Institute of Technology
Department of Fibre and Polymer Technology
Division of Wood Chemistry and Pulp Technology

Stockholm 2008

Fibre and Polymer Technology
Royal Institute of Technology, KTH
SE-100 44 Stockholm
Sweden

AKADEMISK AVHANDLING

Som med tillstånd av Kungliga Tekniska Högskolan framläggs till offentlig granskning för avläggande av teknologie licentiatexamen, torsdagen den 5 juni 2008 kl. 14.00 i STFI-salen, Drottning Kristinas väg 61. Avhandlingen försvaras på engelska.

TRITA-CHE-Report 2008:43
ISSN 1654-1081
ISBN 978-91-7178-985-3

Tryck: Universitetsservice US-AB, Stockholm 2008

Abstract

The accessibility and reactivity of cellulose are key parameters on the manufacturing of cellulose derivatives and regenerated cellulose. It is well known that, due to the crystalline structure of cellulose, the accessibility of solvents and reagents is limited. For instance, an inhomogeneous substitution of the hydroxyl groups of the cellulose chain might lead to the production of derivatives of low quality. As a consequence, part of this work has focused on improving the accessibility and reactivity on cellulose by studying the effect of different monocomponent endoglucanases. It has been demonstrated that the presence of the cellulose-binding domain plays an important role on the enhancement of cellulose reactivity; however, the structure of the catalytic domain has been showed to have the highest influence on this parameter. Furthermore, the influence of mechanical treatment prior to enzymatic treatment has been examined. The combination of pretreatments showed a positive effect enhancing to a larger extent the cellulose reactivity.

Currently, dissolving-grade pulps are commonly used for the production of cellulose derivatives and regenerated cellulose. The requirements for these so-called “special pulps” are high cellulose content and a low hemicelluloses and lignin content. As a result of these specific demands, the production costs of these pulps are higher than those of common kraft pulps. The second part of this work, therefore, has been focused on the study on the viability of converting kraft pulps into dissolving pulps. It has been demonstrated that the combination of enzymatic treatments using a monocomponent endoglucanase and a xylanase together with the addition of an alkaline step could fulfil the requirements of a commercial dissolving pulp in terms of cellulose reactivity and cellulose and hemicellulose content.

Sammanfattning

Cellulosans tillgänglighet och reaktivitet är nyckelparametrar vid framställning av regenererad cellulosa och cellulosaderivat. Det är välkänt att på grund av cellulosans kristallina struktur är tillgängligheten begränsad för lösningsmedel och olika reagens. Till exempel kan en inhomogen substitution av hydroxylgrupperna i cellulosakedjan resultera i cellulosaderivat av sämre kvalitet. Baserat på detta har en del av arbetet i denna studie fokuserat på att förbättra cellulosans tillgänglighet och reaktivitet genom att studera effekten av olika enzymatiska behandlingar med monokomponent endoglukanaser. Resultaten visar att närvaron av en cellulosabindande domän fyller en viktig funktion för att öka cellulosans reaktivitet, men strukturen för den katalytiska domänen visade sig ha den största inverkan på cellulosans tillgänglighet. I kompletterande studier har även effekten av en mekanisk förbehandling i kombination med enzymatisk behandling utvärderats. Kombinationen av förbehandlingarna resulterade i en positiv effekt, cellulosans reaktivitet kunde ökas i större omfattning.

I dag används huvudsakligen dissolvingmassor som råvara vid framställning av cellulosaregenerat och cellulosaderivat. Kraven för dessa s.k. specialmassor är högt cellulosainnehåll samt lågt hemicellulosa- respektive lignininnehåll. På grund av dessa specifika krav är produktionskostnaderna för dessa massor högre än konventionella sulfatmassor. Den andra delen av studien har därför fokuserat på möjligheten att använda dessa sulfatmassor som dissolvingmassa. Det har visats att kombinationen av enzymatiska behandlingar med monokomponent endoglukanas och xylanas följt av ett alkaliskt steg kan resultera i massor där kraven uppfylls med avseende på cellulosans reaktivitet, och cellulosa- respektive hemicellulosainnehåll.

List of Publications

This thesis is based on the following papers that are appended at the end. They will be referred to with roman figures:

I. **The effect of different monocomponent endoglucanases on cellulose accessibility in dissolving pulps**
Viviana Köpcke, Hiroki Nanko and Monica Ek
Manuscript

II. **Increasing accessibility and reactivity of paper grade pulp by enzymatic treatment for use as dissolving pulp**
Viviana Köpcke, David Ibarra and Monica Ek
Submitted to Nordic Pulp and Paper Research Journal

Author contribution

Paper I: Principal author, planned and performed most of the experimental work. The mechanical treatment of the pulps was performed in cooperation with Dr. Hiroki Nanko.

Paper II: Principal author, planned and performed the experimental work together with Dr. David Ibarra.

Results from the above publications have been presented at:

Influence of Mechanical and Enzymatic Treatment on Cellulose Accessibility
Hermosilla, V., Nanko, H. and Ek, M.
14th ISWFPC: International Symposium on Wood, Fibre and Pulping Chemistry, June 25-28, Durban, South Africa, 2007. Conference Proceedings, abstract 117.

Study on the feasibility of converting kraft pulps into dissolving pulps: accessibility and reactivity
Köpcke, V., Ibarra, D. and Ek, M.
235th American Chemical Society (ACS) National meeting, April 6-11, New Orleans, USA, 2008. Abstract of papers CELL-257.

Table of contents

1 Introduction

1.1 Purpose of the study

The accessibility of cellulose to solvents and reagents is of great importance in the manufacturing of cellulose derivatives and regenerated cellulose. A homogeneous substitution of the hydroxyl groups in the cellulose chain is desired in order to obtain products of high value; however, the compact structure of cellulose makes its accessibility difficult, leading to an inhomogeneous substitution. Dissolving-grade pulps are mainly used as raw material for the production of cellulose derivatives and regenerated cellulose. But, because of specific requirements (i.e., high cellulose content and very low amounts of hemicelluloses), the cost of these pulps is higher than common paper-grade pulps.

The purpose of this work is, therefore, to enhance the accessibility of cellulose and improve the reactivity of different pulps. The effects of enzymatic, mechanical, and chemical treatments are investigated individually and in combination. Furthermore, the feasibility of converting two different hardwood kraft pulps into dissolving pulps is examined.

1.2 Background

1.2.1 Cellulose

Cellulose is a linear polymer that consists of β(1→4) linked D-glucose units. An individual glucose unit within the chain presents a rotation of 180° with respect to the following glucose unit and within the cellulose chain; two linked glucose units form a cellobiose unit. At the end of the cellulose chain, two different terminal hydroxyl groups are found: the non-reducing end, which is conformed by an alcoholic hydroxyl group, and the reducing-end, which presents an aldehyde hydrate group [1]. The structure of cellulose is illustrated in *Fig 1*.

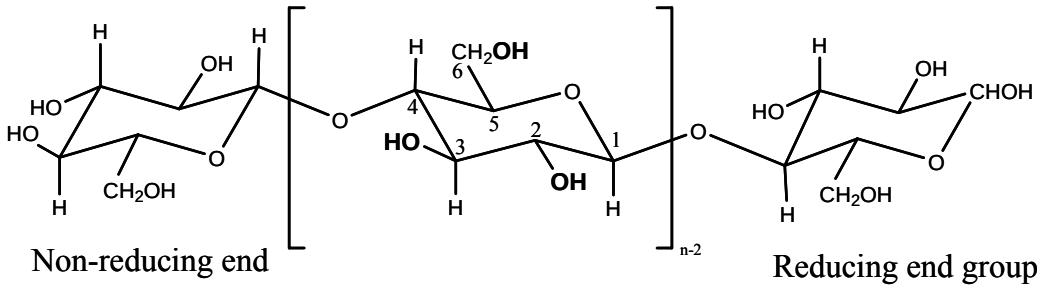

Fig 1. Cellulose structure [1].

There are three reactive hydroxyl groups (C2, C3, and C6) in each glucose unit. When these groups interact with the cellulose molecules, the formation of intramolecular hydrogen bonds (interaction of the hydroxyl groups within the same molecule) and intermolecular hydrogen bonds (interaction of the hydroxyl groups with neighbouring cellulose molecules) take place. By the arrangement of these bonds, mainly intramolecular bonds, the cellulose molecules form a stiff network, giving rise to the close compact structure of cellulose. This structure is not uniform, and both highly ordered (crystalline) regions and regions with a low degree of order can be found. The relative proportion of these regions depends on the raw material and the treatments to which the cellulose has been subjected [2]. Moreover, these regions are responsible for the limited solubility of cellulose and make it difficult for solvents and reagents to access areas within the cellulose fibres.

1.2.2 Cellulose derivatives and regenerated cellulose

Today, the production of cellulose is mainly focussed on the pulp and paper industry. However, a branch of it involves the manufacturing of cellulose derivatives and regenerated cellulose. In the production of cellulose derivatives, cellulose is modified by substitution at the hydroxyl groups, whereas for regenerated cellulose, cellulose is chemically dissolved and then regenerated. Examples of cellulose derivatives are: carboxymethylcellulose (CMC), which is used as a thickener and stabilizer; ethylhydroxyethylcellulose (EHEC), which is used as a water-retaining agent and as a thickener; and cellulose nitrate, which is used in the production of explosives. The main product of regenerated cellulose is rayon, which is used in the textile industry. These cellulose-based products are useful due to their specific solubilities, flexibility, and softness [3]. Their applications are concentrated within the pharmaceutical, textile, food, and painting industries, among others.

1.2.3 Regenerated cellulose: The viscose process

The viscose process is a process widely used for the production of regenerated cellulose (mainly rayon). The process, which was developed in the 1800s, involves the reaction of cellulose with sodium hydroxide to form alkali cellulose followed by the addition of carbon disulphide (CS_2) to produced cellulose xanthate. The cellulose xanthate is then dissolved in diluted sodium hydroxide to generate a thick solution, which is called viscose. To regenerate the cellulose, the solution is ripened, extruded through spinnerets, and immersed acid bath where regeneration occurs.

When viscose filaments are produced, it is desirable to have a homogeneous product. However, clogging of the viscose filters often presents a problem. To avoid this problem, a maximum dissolution of cellulose is desirable. This can be achieved by increasing the amount of carbon disulphide. Unfortunately, CS_2 is an expensive and

highly toxic (malodorous, highly volatile, and flammable) liquid with harmful effects on the environment [4,5]. Consequently, several studies have been aimed at developing methods of reducing the consumption of carbon disulphide while at the same time achieving maximum cellulose activation, for a further modification. Furthermore, alternative solvents have been successfully tested, presenting environmentally acceptable conditions at higher costs. An example of this is N-methylmorpholine-N-oxide (NMMO), which is used for the production of Lyocell [6].

1.2.4 Cellulose accessibility and reactivity

The accessibility and reactivity of the cellulose structure has been a topic of high interest. As mentioned above, the accessibility within the cellulose fibres is limited by the compact structure of cellulose, which is determined by the presence of highly ordered regions formed by strong hydrogen bonds [7]. As a consequence, the most complicated challenge in the production of regenerated cellulose is to achieve the complete dissolution of the cellulose structure; unfortunately, this cannot be effected with cheap and common solvents. For this reason, it is important to enhance the cellulose accessibility and reactivity in order to obtain homogeneous products. The enhancement may also decrease the amount of carbon disulphide required in the viscose process.

It has been proposed that the accessibility of cellulose depends mainly on the number and size of the pores in the cellulose structure; the size and type of solvent or reagent; the internal surface, as determined by the size of fibrils or fibril aggregates, that is accessible; and the structure of the cellulose molecules, which will determine which hydroxyl groups are accessible. Therefore, to increase cellulose accessibility, the pores must be opened, and both the fibril aggregates and the highly ordered regions must be altered [1].

In recent years, several studies have proposed optimal pretreatments, alone or in combination, to increase cellulose accessibility and reactivity. Among them, chemical, mechanical, and enzymatic treatments have been tested. Furthermore, enzymatic treatment has become a topic of great interest since enzymes are non-toxic and environmentally harmless.

1.2.5 Chemical treatments

The main purpose of subjecting the cellulose to chemical treatment is to increase the swelling of the cellulose fibres with different solvents. When the fibres swell, several hydrogen bonds are broken due to the high stress caused by swelling. As a result, the cellulose structure becomes less ordered – in some cases the highly ordered regions are completely disrupted – leading to an increase of the active surface area, i.e., an

increase in the number of available hydroxyl groups and, therefore, the accessibility to solvents [8]. Studies of the effect of chemical treatments on the activation of the cellulose structure include, among others, the use of different aqueous and non-aqueous solvents [9, 10], the use of sodium hydroxide as a swelling solvent [10, 11], and the combination of sodium hydroxide and urea [12].

1.2.6 Mechanical treatments

Mechanical treatment of the cellulose fibres is used in the pulp and paper industry because of its capacity to enhance fiber-fiber bonding, to cut or make the fibres stronger, and to produce changes on the cellulose structure. For instance, strong bonds among fibres gives the printing paper strong and smooth properties [13].

When the pulp is subjected to mechanical treatment, the interfibrillar bonds, which are mainly located in the primary wall and in the outer lamella of the S1 layer of the cell wall, are disrupted. This effect leads to an increase in the reactive surface area of the fibres, improving the accessibility of the cellulose [1]. In several studies, mechanical treatment has been used in combination with other treatments [14, 15].

1.2.7 Enzymatic treatments

Enzymes have broad industrial applications. They have been used in the detergent, food, and pharmaceutical sectors. Enzymes have also been studied in the pulp and paper industry [16-18], and they are currently used for several applications, including deinking and as bleaching agents [19]. The effect of enzymatic treatments on cellulose reactivity has also been investigated. It has been reported that enzymatic treatments, especially cellulases on dissolving pulps, hold a great potential for increasing cellulose reactivity [20-25].

Cellulases: monocomponent endoglucanases.

Cellulases are enzymes that hydrolyse the 1,4-β-D-glucosidic bonds of the cellulose chain. There are three major groups of cellulases: endoglucanases, cellobiohydrolases or exoglucanases, and glucosidases as illustrated in *Fig 2*. These enzymes can act alone on the cellulose chain or together. When they act together, a synergistic phenomenon is often generated, resulting in an efficient degradation of the cellulose structure.

Endoglucanases are enzymes that randomly cleave the amorphous sites of the cellulose creating shorter chains (oligosaccharides) and, therefore, new chain ends. Cellobiohydrolases or exoglucanases attack the reducing and non-reducing ends of the

cellulose chains, generating mainly glucose or cellobiose units. This type of cellulase can also act on microcrystalline cellulose by a peeling mechanism. Glucosidases act on cellobiose generating glucose units [26]. It has been suggested that there are three primary parameters affecting the degree of enzymatic hydrolysis: the crystallinity, the specific surface area, and the degree of polymerization of the cellulose [27, 28].

Most cellulases consist of two domains. The first is a catalytic domain, which is responsible for the hydrolysis of the cellulose chain. The catalytic domain of endoglucanases is "cleft-shaped." Exoglucanase, on the other hand, have a "tunnel-shaped" catalytic domain structure. The second is a cellulose-binding domain (CBD), which helps the enzyme to bind to the cellulose chain bringing the catalytic domain close to the substrate. An interdomain linker serves as a connections between the two domains [29].

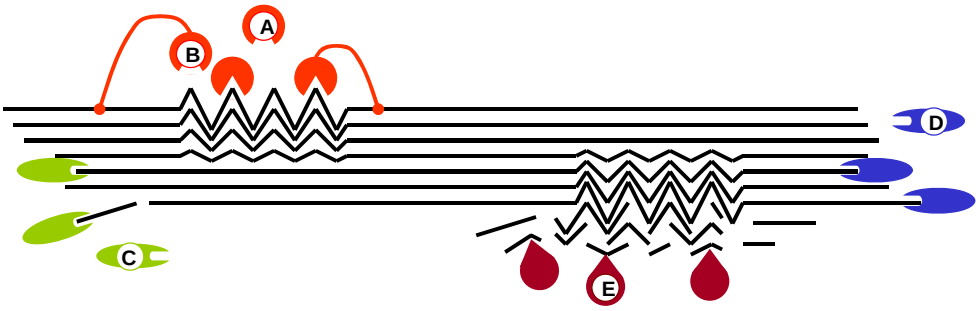

Fig 2. Types of cellulases: (A) endoglucanases without cellulose-binding domain; (B) endoglucanases with cellulose-binding domain; (C) and (D) cellobiohydrolases, and (E) glucosidases.

1.2.8 Reactivity measurements

Several methods have been developed to measure cellulose reactivity, including: iodine sorption [30], water retention value [31], swelling water coefficient, viscose filter value (which is complicated, requiring special equipment) [32], and the Fock method (which simulates the viscose process on a laboratory scale and is easier to handle) [33]. The Fock method was used in this work and is explained in the experimental part (*Sec. 2.1.6*).

1.2.9 Dissolving-grade pulps

For regenerated cellulose manufacturing, it is generally known that the raw material is required to have a high cellulose content (over 90%) and low levels of hemicellulose, lignin, extractives, and minerals.

Today, the raw materials used for the production of regenerated cellulose are dissolving-grade pulps and, to a lesser extent, cotton linters. Dissolving-grade pulps are produced mainly by two different processes: the sulphite process and the

prehydrolysis kraft process. Other pulping processes have been investigated for the production of these pulps, including organosolv pulping [34, 35]. This process is based on the use of organic solvents; however, the expense of solvent recovery is the biggest drawback of this process.

Dissolving pulps present higher costs than kraft pulps. This can be attributed to several factors, such as wood costs (the production of these pulps has a lower yield since hemicelluloses are dissolved and washed away); capital costs (because the yield is low, more equipments may be needed to have a high production); chemical costs; production rates (lower than for paper-grade pulps); and the inventories and storage space (the pulps are produced for specific customers with certain requirements, which implies a high control of the inventory) [36]. As a consequence, the viability of converting paper-grade pulps into dissolving pulps arises.

1.2.10 Converting paper-grade pulps into dissolving-grade pulps

In recent years, several studies have used different methods to examine the feasibility of modifying paper-grade pulps for further use as dissolving pulps. These studies have focused mainly on the optimal removal of hemicelluloses because in the production of viscose, hemicelluloses can affect the viscose filterability, the xanthation of cellulose, and the strength of the end product [37].

Several methods have been reported for the removal of hemicelluloses, including treatments with alkaline extraction [38], nitren and cuen extraction [39, 40], and a combination of pretreatments using xylanases and alkaline extraction [41]. However, little attention has been paid to the effect of changes in the accessibility and reactivity of the cellulose after these treatments.

2 Materials and Methods

An overview of the materials and methods used in this work is given. More detailed information is found in papers I and II.

2.1 Pulps

Bleached commercial dissolving softwood pulp from a mixture of Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) with a lignin content of 0.6% and R18 > 94%, provided by Domsjö Fabriker AB Sweden, was investigated. Additionally, two ECF bleached hardwood kraft pulps from birch (*Betula pendula*), kappa number 0.7, ISO brightness 90%, and viscosity 770 dm^3/kg provided by Metsä Botnia (Finland), and eucalypt (*Eucalyptus globulus*), kappa number 0.9, ISO brightness 91%, and viscosity 860 dm^3/kg provided by Ence (Spain) were use in this work. As a reference pulp, a commercial ECF bleached dissolving pulp from eucalypt (*Eucalytpus globulus*) provided by Sniace (Spain) was considered. The characteristics of the pulp were ISO brightness 91%, viscosity 530 dm^3/kg, α-cellulose 92%, and hemicellulose content 2.4%.

The dissolving pulp from softwood was a never-dried pulp. The kraft pulps and the dissolving pulp from hardwood were obtained in dried sheet forms. Previously to the treatments, the dried sheets were soaked in water for 24 h and disintegrated in an L&W instrument at 1.5% consistency at 30 000 revolutions following the standard ISO 5263-1:2004.

.

2.2 Enzymes

Three different commercial monocomponent endoglucanases were examined. The enzymes are all produced from a genetically modified *Aspergillus* species but they differ either in the presence of a cellulose-binding domain (CBD) or in the structure of the catalytic domain. One is a monocomponent endoglucanase (EGV) with a CBD, and the other two are monocomponent endoglucanases (EGV and EGI) without CBDs. Their cellulolytic activity was determined by the manufacturer and expressed in Endo-cellulose Units (ECU) per unit mass of material. The EGV with a CBD and the EGV without a CBD have the same cellulolytic activity of 5000 ECU/g. The EGI without a CBD has a cellulolytic activity of 2500 ECU/g.

Table 1. Characteristics of the different monocomponent endoglucanases

Name	Enzyme	Cellulose-binding domain	Enzymatic activity (ECU /g)
N476	EG V	Yes	5000
N613	EG I	No	2500
N51063	EG V	No	5000

A commercial xylanase was also used. This enzyme is produced from a genetically modified *Bacillus* species. The xylanase activity was determined by the manufacturer and expressed in Endo-xylanase Units (EXU) per unit mass of material as 1000 EXU/g.

2.3 Enzymatic treatments

The enzymatic treatments were performed at pH 7 with a 3% pulp consistency. A phosphate buffer (11 mM NaH_2PO_4 and 9 mM Na_2HPO_4) was used. To carry out the treatment, the buffer solution containing the enzyme was added the pulp in order to obtain a homogeneous mixture. The treatment was performed in sealed plastic bags, which were immersed in a water bath at 50 ºC for the endoglucanase and at 60 ºC for the xylanase. To obtain a uniform distribution, the pulps were kneaded every 10-30 minutes, depending on the incubation time. To denaturize the enzyme, the mixture was treated with hot water and filtered through a Büchner funnel. Deionised water at 90 ºC was added to the mixture and the bags placed in a 90 ºC water bath for 30 minutes. The mixture was finally filtered and washed with deionised water.

It must be pointed out that a different batch was used for the study of the incubation time of N613 and for the analysis of the influence of the enzyme structure (catalytic and cellulose-binding domain) on cellulose reactivity (*Sec. 3.1.3*).

2.4 Mechanical treatments

Mechanical treatments were performed using a laboratory grinder (Cerendipitor MKCA6-3, Masuko Sangyo Co., Ltd., Japan). The grinder features two nonporous ceramic grinding discs with an adjustable clearance between the upper and lower discs. While the upper grinding disc is fixed, the lower one is rotated at a high speed. Raw materials, fed into the hopper, are dispersed by centrifugal force into the gap between the upper and lower grinding discs, where they are ground by massive compressive, shearing, and rolling friction forces.

2.5 Chemical treatment

The kraft pulps were subjected to an alkaline extraction using a 9% solution of NaOH at room temperature for 1 h. The pulp had a consistency of 4%.

2.6 Reactivity measurements

Cellulose reactivity was measured using a slightly modified Fock method [33]. This method, similar to a microscale viscose process, measures the amount of regenerated cellulose. The procedure is explained below:

Half a gram of dry pulp was mixed with 50 ml of 9% NaOH and 1.3 ml of CS_2 in a 100 ml Erlenmeyer flask with a stopper. The sample was stirred for 4 hours at room temperature and diluted to 100 g with deionised water. The solution was then carefully shaken and left for 2 hours to allow the undissolved cellulose to settle to the bottom of the flask. Subsequently, 10 ml taken from the upper part of the solution (dissolved cellulose), was transferred to a 100 ml Erlenmeyer flask and neutralized with 20% H_2SO_4. The sample was left overnight at room temperature. An aliquot of 20 ml of 68% H_2SO_4 was added to the regenerated cellulose, and the sample was stirred for 1 h. The solution was diluted with 50 ml of deionised water, and 10 ml of 1/6 M $K_2Cr_2O_7$ was added. The mixture was agitated and heated for 1 h to oxidise the regenerated cellulose. The solution was transferred to a 100 ml flask and diluted to 100 ml. 40 ml of the solution was poured into a 250 ml beaker that contained 0.5 g of KI. The mixture was titrated with 0.1% Na_2SO_4, using starch as an indicator. The titration was stopped when all of the I_2 was reduced, i.e., when the colour of the solution turns light blue.

The amount of regenerated cellulose was calculated using the following equation (1):

$$\%\ \text{Reacted cellulose} = (100) * 9.62 \frac{M\left[V_1C_1 - \left(V_2C_2\left(\frac{100}{40}\right)\left(\frac{1}{6}\right)\right)\right]}{4m} \qquad (1)$$

where M is the molecular mass of glucose (162 g/mol), V_1 is the volume of $K_2Cr_2O_7$ added (0.010 L), C_1 is the concentration of $K_2Cr_2O_7$ (mol/L), V_2 is the volume of Na_2SO_4 consumed during the titration, C_2 is the concentration of Na_2SO_4 (0.1 mol/L), m is the weight of the sample (g), 9.62 is calculated from the first dilution of the sample to 100 g and the aliquot of 10 ml (10.4 g) taken from the dissolved part, so (100/10.4) = 9.62, and (100/40) is calculated from the dilution of the sample after oxidation (100 ml) and the aliquot taken for the titration with Na_2SO_4 (40 ml).

All the measurements were carried out in triplicates and the average value was considered.

2.7 Viscosity

Viscosity was estimated according to SCAN-CM 15:99 (ISO 5351).

2.8 Pulp yield

The yield was determined gravimetrically. The sample was weighted before and after the treatments, and the difference in weight was taken to be a loss in yield.

2.9 Fourier transform-infrared spectroscopy (FTIR)

FTIR spectra were recorded on a Perkin-Elmer 2000 FTIR spectrometer. The wave number range scanned was 4000-700 cm^{-1}.

2.10 Carbohydrate analysis

The carbohydrate compositions of the pulps were analyzed after acid hydrolysis, reduction with sodium borohydride, and subsequent acetylation [42]. The samples were examined by gas chromatography (GC) using a Hewlett-Packard HP-6890 chromatograph.

3 Results and Discussions

3.1 Behaviour of two monocomponent endoglucanases with and without a cellulose-binding domain (CBD) on dissolving pulp (Paper I)

3.1.1 Enzymatic treatments

Reactivity and viscosity

In order to improve cellulose reactivity, dissolving pulp was subjected to enzymatic treatment using two different commercial monocomponent endoglucanases, N476 and N613, with and without the presence of CBD, respectively. The behaviour of both enzymes was examined in terms of reacted cellulose and viscosity. The results are presented in *Fig 3*.

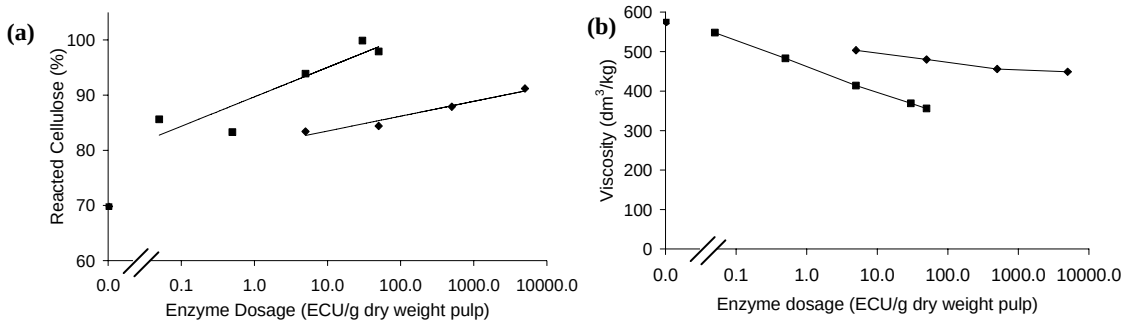

Fig 3. **(a)** Reactivity, according to Fock, after enzymatic treatment. **(b)** Pulp viscosity after enzymatic treatment. The enzymatic treatments were done with: (■) a monocomponent endoglucanase with a CBD (N476) and (♦) a monocomponent endoglucanase without a CBD (N613). Incubation time: 1 h. Enzyme dosages for N613: 0, 5, 50, 500, and 5000 ECU/g dry weight pulp. Enzyme dosages for N476: 0, 0.05, 0.5, 30, and 50 ECU/g dry weight pulp. The values for N476 were taken from a previous study [22].

It is shown in *Fig 3a* that cellulose reactivity was enhanced by both enzymes but in different degrees. Cellulose reactivity was enhanced by about 20%-points using N613, although it was noticed that even at high enzyme dosages (5000 ECU/g dry weight pulp), the reactivity did not reach values higher than 90%. In contrast, it was demonstrated in a previous study that N476 could increase the reactivity to 100%, even at low dosages (30 ECU/g dry weight pulp) [22].

The lack of a CBD and the different catalytic domain presented by N613 may explain this result. Firstly, the CBD facilitates the binding of the enzyme to the substrate. Secondly, it has been suggested that endoglucanases attack amorphous areas rather than crystalline areas [29] and that an EG I (N613) presents a lower catalytic rate towards low ordered cellulose than EG V (N476) [43].

The pulp viscosity showed a lower decrease after treatment with N613. This supports the lower enhancement in reactivity, as presented in *Fig 3b*. In contrast, pulp treated with N476 presented a more marked decrease in viscosity, as expected.

.

Enzyme incubation time

The optimization of the incubation time of the enzyme was measured at an enzyme dosage of 30 ECU/g dry weight pulp. It was observed that the enzyme reacted rather quickly; the reactivity was constant after 30 minutes. A similar behaviour was noticed with N476, which had a reaction time of 10 minutes [22].

Pulp yield

After enzymatic treatment with N613, the pulp was recovered in 99% yield. The same result was obtained with N476.

3.1.2 Mechanical treatment

To further improve the cellulose reactivity with N613, mechanical treatment was added prior to enzymatic treatment to increase the reactive surface of the fibrils by grinding. The fibrillation effect of this treatment was examined by light microscopy, as shown in *Fig 4*.

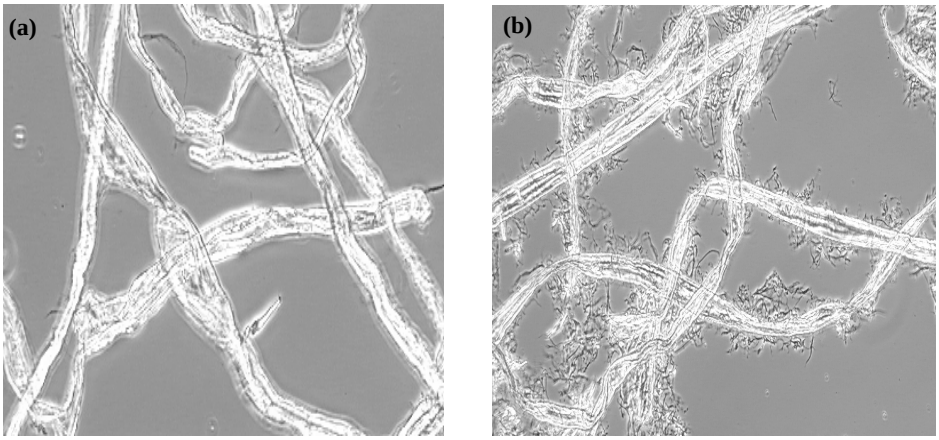

Fig 4. Commercial dissolving pulp: **(a)** untreated, and **(b)** after being subjected to an intensive mechanical treatment.

Reactivity and viscosity

After intensive mechanical treatment, the reactivity of the dissolving pulp was greater than that of the pulp that was subjected only to enzymatic treatment. The improvement in reactivity was about 10%-points with respect to the control pulp, as shown in *Fig 5a*. This improvement can be explained by the fibrillation that occurs during mechanical treatment, which mainly affects the outer layers of the cell wall (primary wall and outer lamella of the S1), leading to a larger available surface area [1]. Moreover, the fibrillation effect may facilitate the entry of enzyme into the fibres, resulting in a better performance and contributing to the improvement in the cellulose reactivity.

It was observed that the pulp subjected only to mechanical treatment had an enhanced reactivity to that of a pulp treated with enzyme at around 50 ECU/g dry weight pulp. However, the addition of mechanical treatment prior to the addition of N613 was not as effective as the enzymatic treatment using only N476, which reached a reactivity value of 100%, as reported earlier [22].

Furthermore, the addition of mechanical treatment slightly affected the pulp viscosity, as illustrated in *Fig 5b*. The decrease in viscosity was more marked at higher enzyme dosages, which suggests that the enzymatic treatment at these conditions is enhanced by the mechanical treatment.

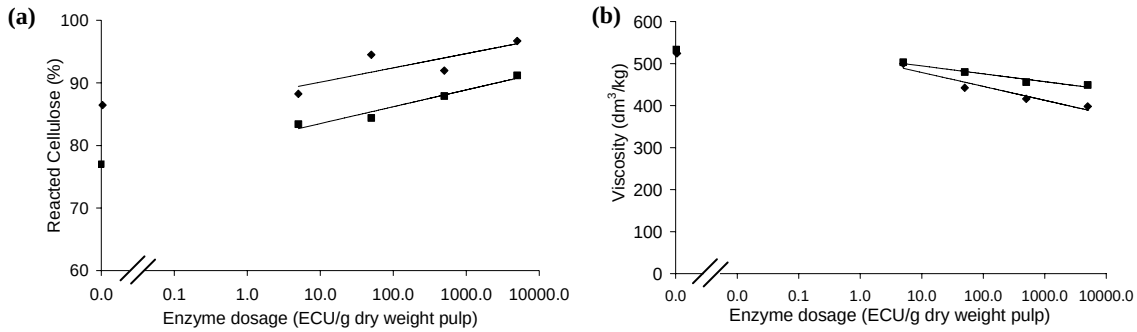

Fig 5. **(a)** Reactivity, according to Fock, of the treated pulp. **(b)** Viscosity of the treated pulp. The pulp was subjected to (■) enzymatic treatment with N613 only and (♦) intensive mechanical treatment followed by enzymatic treatment with N613. Enzyme dosage: 0, 5, 50, 500, and 5000 ECU/g dry pulp. Incubation time: 1 h.

Enzyme incubation time

After the addition of mechanical treatment, the enzyme showed an immediate response towards cellulose. This is in contrast to the slower response (30 minutes) of the enzyme alone, as shown in *Fig 6*. This positive effect may be due to the increase of available fibril surfaces after mechanical treatment.

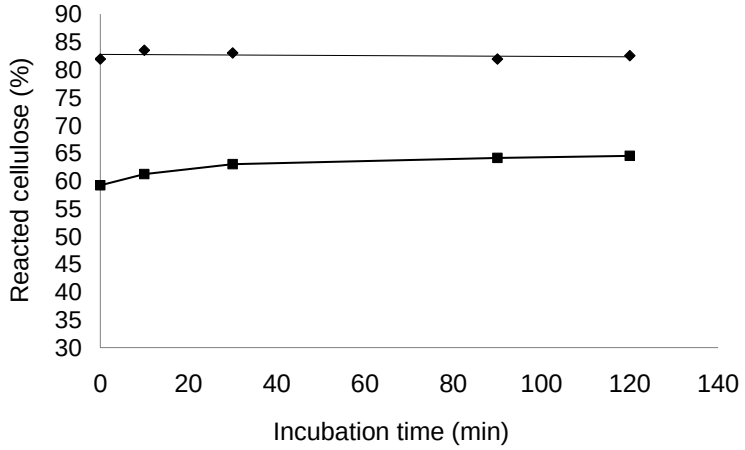

Fig 6. Reaction time of N613 towards the dissolving pulp with: (■) only enzymatic treatment and (♦) an intensive mechanical treatment followed by enzymatic treatment. Enzyme dosage: 30 ECU/g dry weight pulp.

Pulp yield

The pulp yield decreased to 90% after the addition of mechanical treatment. The loss in yield may be attributed to i) the formation of fines (due to the fibrillation of the cell wall), which are lost during the filtration of the pulp and ii) to the loss of pulp during the treatment, since the pulp passed through the grinder 11 times.

3.1.3 Influence of the enzyme structure on cellulose reactivity

As demonstrated previously, N613 could not enhance the cellulose reactivity as much as N476 in spite of the addition of mechanical treatment, which improved the reactivity. This response may be mainly due to the lack of a cellulose-binding domain, the difference in the catalytic domain, or both effects. In order to determine which parameter has the greatest influence on the enhancement of cellulose reactivity, a third enzyme – N51063 – with the same catalytic domain as N476 but without a cellulose-binding domain was included in this study. The three enzymes were compared and presented in *Fig 7*.

Considering only the effect of the cellulose-binding domain, N476 and N51063 were compared. The two enzymes showed approximately the same improvement in reactivity when the enzyme dosage was increased in comparison to the control pulp.

Therefore, the presence of a cellulose-binding domain affected the cellulose reactivity in some extent, which is mainly due to the fact that the cellulose-binding domain of N476 facilitates the cleavage of the cellulose chain by approximating the catalytic domain of the enzyme. Nevertheless, in the case of N613, the cellulose reactivity was improved but not to the same degree as in the cases of the other two enzymes. This suggested that, even though this enzyme lacks a cellulose-binding domain and has a different catalytic domain, the latter seems to have a greater impact on the improvement in cellulose reactivity. It has been proposed that endoglucanases, in general, have a higher affinity for low ordered cellulose. It was proposed that, while the presence of a cellulose-binding domain does not influence significantly on the activity of the enzyme towards amorphous cellulose, it does influence the activity in crystalline cellulose [44], which confirms the major effect of the catalytic domain on cellulose reactivity.

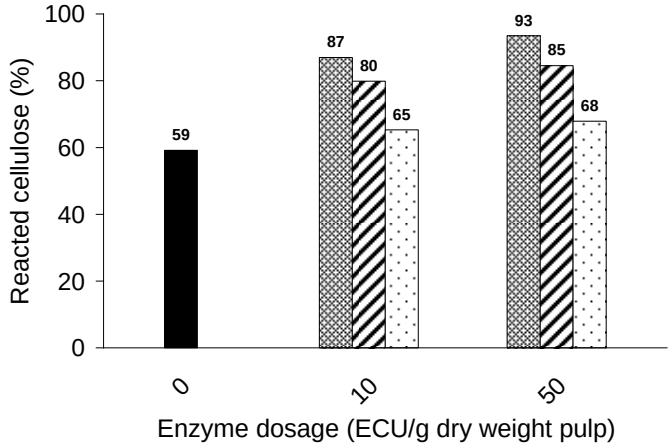

Fig 7. Comparison of the cellulose reactivity, according to Fock, among N476 (⊞), N51063 (▨), N613 (⊡) and a control pulp (■). Incubation time: 1 h.

From these results, it can be concluded that not all monocomponent endoglucanases display the same effects towards cellulose reactivity. The cellulose reactivity also depends on the substrate, which may present different characteristics regarding the raw material and the process from which the pulp was manufactured.

3.2 Feasibility of converting paper-grade pulp into dissolving pulp (Paper II)

It has been shown that a monocomponent endoglucanase with a cellulose-binding domain, in this case N476, presented the greatest improvement on cellulose reactivity towards a dissolving pulp. This suggested that it might be valuable to study the behaviour of the same enzyme, in terms of cellulose reactivity, towards a commercial kraft pulp in order to investigate its viability as a dissolving pulp.

By definition, dissolving pulps must contain high cellulose content and low levels of hemicellulose, lignin, extractives, and minerals; however, paper-grade pulps contain relatively high cellulose content, but they also contain a large amount of hemicelluloses. For this reason, a reduction in the hemicelluloses content must also be achieved.

3.2.1 Enzymatic treatment

Reactivity and viscosity

The positive effect on cellulose reactivity, according to Fock, shown by the monocomponent endoglucanase towards the dissolving pulp was also observed after treating birch and eucalypt kraft pulps with the same enzyme. As shown in *Fig 8a*, for birch pulp, the reactivity reached the same values as the commercial dissolving pulp (64%) at an enzyme dosage of 250 ECU/g dry weight pulp. At the same enzyme dosages, eucalypt pulp showed a reactivity value of 60%, nearly at the level of the commercial dissolving pulp. It was noticed that, at higher dosages, a very small increase in reactivity was observed. This may be explained by the high hemicellulose content in both kraft pulps. Hemicelluloses may limit the access of the endoglucanase to areas within the fibres. Additionally, the size of the enzyme may also be an obstacle to entering into areas with stretched bonds, which may reduce the area available for cleavage.

Viscosity was also examined. Both pulps displayed a decrease in viscosity when the enzyme dosage was increased, as shown in *Fig 8b*. This decrease is mainly attributed to the cleavage of the cellulose chain performed by the endoglucanase. The same effect on dissolving pulp and softwood kraft pulp has been reported previously [17, 22, 25]. Following the same tendency as in the reactivity, the birch pulp showed a more marked decrease in viscosity than the eucalypt pulp, which can also be explained by the differences in structure of the pulps, e.g., crystalline regions.

However, for both pulps, the major decreases in viscosity were observed before an enzyme dosage of 50 ECU/g dry weight pulp was reached. After this dosage, the viscosity decreased disproportionately less.

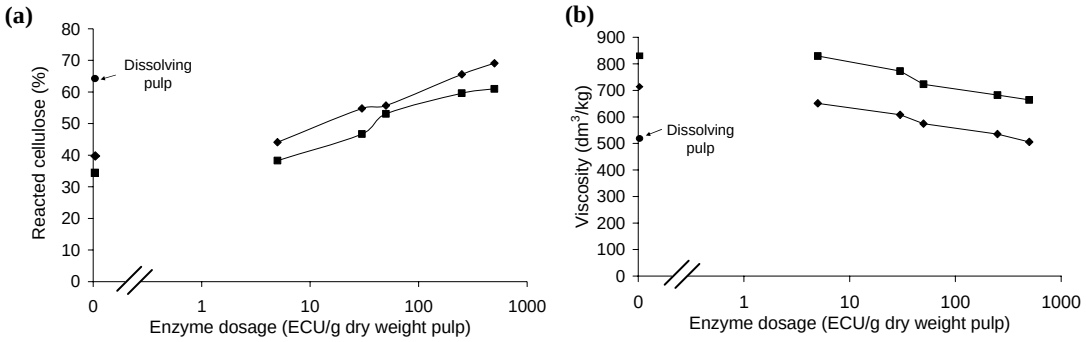

Fig 8. **(a)** Reactivity, according to Fock, of the treated pulps. **(b)** Viscosity of the treated pulp. The (♦) represents the birch pulp and the (■) represents the eucalypt pulp. Enzyme dosage: 0, 5, 50, 500, and 5000 ECU/g dry weight pulp. Incubation time: 1 h.

Enzyme incubation time

It is shown in *Fig 9* that both pulps demonstrated the same tendency in reactivity; i.e., for both pulps, the highest increase in reactivity occurred during the first 15 minutes. For birch, after 30 minutes reactivity reached a constant value. However, for eucalypt, the reactivity continued, slightly increasing after 30 minutes. The response of the enzyme in eucalypt pulp suggests that this pulp may contain more aggregated fibrils, which may cause the slower reaction with the enzyme.

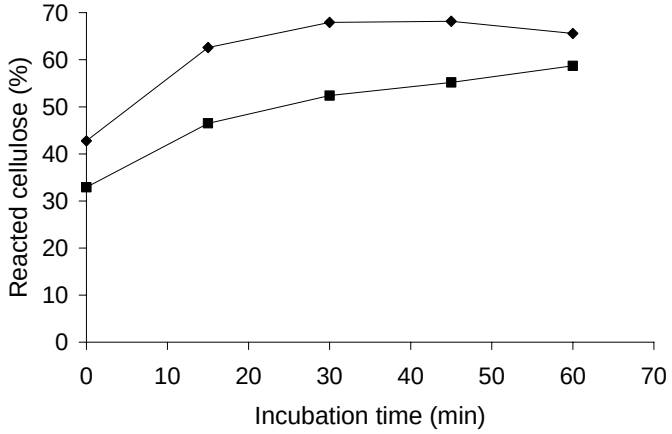

Fig 9. Reactivity, according to Fock, of the birch-treated pulp (♦) and eucalypt-treated pulp (■) as a function of the incubation time. Enzyme dosage: 250 ECU/g dry weight pulp.

Structural analysis by FTIR

The structural analysis by FTIR of both enzyme-treated pulps is illustrated in *Fig 10a* and *Fig 10b*. The spectra show typical cellulose peaks for both cases, which are seen around 1000-1200 cm^{-1} [45]. In previous studies on dissolving pulps treated with endoglucanases, a change on the intermolecular and intramolecular hydrogen bond OH vibration peak of cellulose fibrils at 3300 cm^{-1} was observed [46]. The same change has been noticed after enzymatic treatment of the kraft pulps. The fact that the band became narrower, compared with the control pulp, confirmed that the hydrogen bonds had been altered to some extent and broken by the action of the endoglucanase.

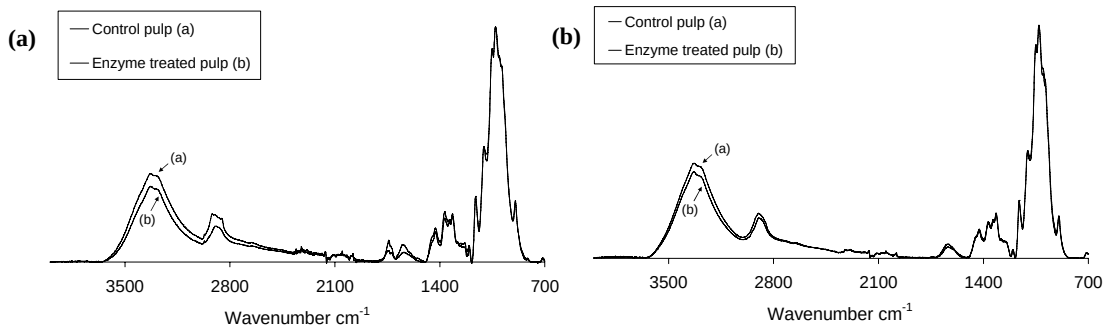

Fig 10. **(a)** FTIR spectrum for birch pulp. **(b)** FTIR spectrum for eucalypt pulp. Enzyme dosage: 250 ECU/g dry weight pulp. Incubation time: 1 h.

3.2.2 Hemicelluloses content

The chemical composition of both paper-grade kraft pulps is shown in *Table 2*. Both pulps present a high amount of hemicelluloses, and as both pulps are from hardwoods, xylan makes the greatest contribution to the hemicellulose content. Consequently, we focused on reducing xylan levels.

Table 2. Chemical composition of the untreated kraft pulps

Pulp	**Glu (%)**	**Man (%)**	**Xyl (%)**	**Ara (%)**	**Gal (%)**
Birch	73.4	<1	25.5	0.4	0.0
Eucalypt	78.6	0.0	21.4	0.0	0.0

Xylanase treatment

Enzymatic treatment with xylanase was performed to decrease the xylan content of the initial pulps. It was noticed (*Fig 11*) that, after enzymatic treatment, the xylan content rapidly decreased, even at low enzyme dosages. Furthermore, at 500 EXU/g dry weight pulp, the remaining xylan in both pulps could not be further reduced, reaching a minimum value of around 13%. This may be due to the limited ability of the enzyme to enter regions within the fibres due to its size. Furthermore, it has been

suggested that, during bleaching, accessible hemicelluloses are dissolved and the hemicelluloses that remain may have been chemically modified, altering their sensitivity to the xylanase [47]. Besides, the hemicelluloses-lignin linkage may also restrict the efficiency of the enzyme [37]

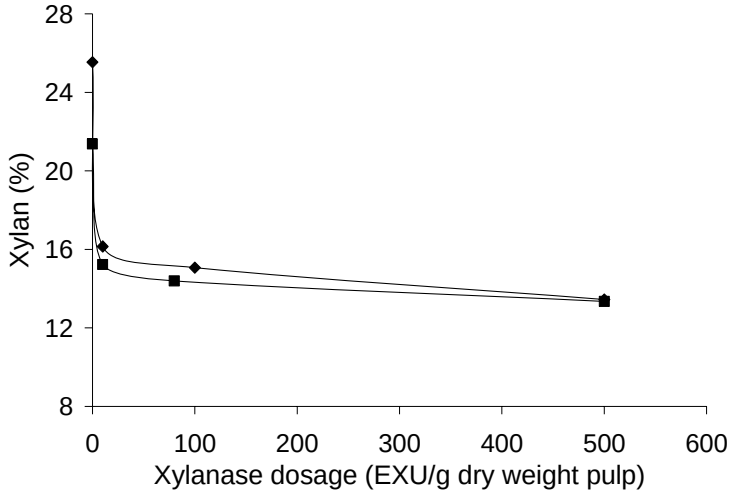

Fig 11. Reduction of xylan content of birch (♦) and eucalypt (■) kraft pulps as a function of the enzyme dosage. Incubation time: 2 h.

3.2.3 Synergy between enzymatic treatments

A combination of enzymatic pretreatments was tested. The addition of xylanase prior to endoglucanase was investigated for both pulps, in terms of viscosity and reactivity according to Fock. A synergistic effect between xylanases and endoglucanases has been reported [48]. However, different responses for the two pulps were obtained after the addition of the enzymatic pretreatments in sequence. For birch, a very slight synergistic effect was observed after the addition of both enzymes, as illustrated in *Fig 12a*. Therefore, the removal of xylan seemed not to enhance the cellulose reactivity and the optimal enzyme dosage remained at 250 ECU/g dry weight pulp.

In *Fig 12b*, it is seen that viscosity showed a decrease after the addition of the xylanase, which suggests that there may be other parameters affecting the cellulose reactivity or that the endoglucanase continued cleaving the already accessible cellulose chains before the xylan was removed.

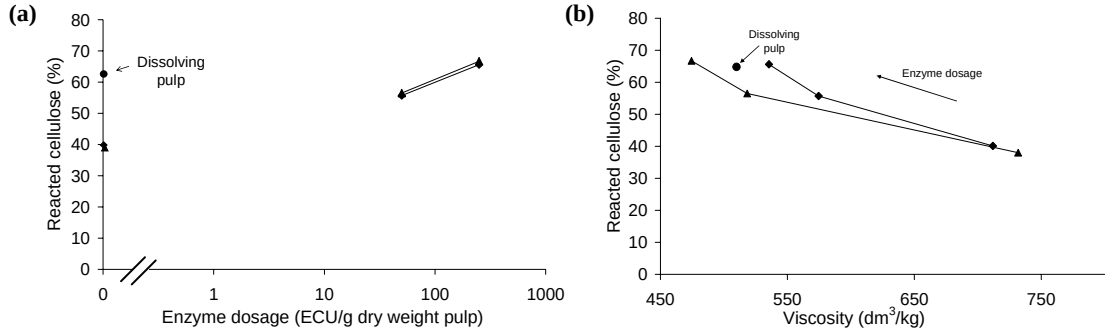

Fig 12. **(a)** Cellulose reactivity of birch pulp as a function of the enzyme dosage and **(b)** viscosity of the pulp after a treatment with: (♦) endoglucanase and (▲) xylanase prior to endoglucanase. Incubation time for endoglucanase: 1 h, and for xylanase: 2 h. Enzyme dosage: 0, 50, and 250 ECU/g dry weight pulp.

In the case of eucalypt pulp, the synergism between the combination of enzymatic pretreatments was clear, as is shown in *Fig 13a*. At an enzyme dosage of 250 ECU/g dry weight pulp, the reactivity reached values higher than that of the commercial dissolving pulp (71%). In this case, the removal of xylan enhanced the available area, i.e., pore size within the fibres, for the endoglucanase to enter and cleave the cellulose chain. Viscosity, as illustrated in *Fig 13b*, showed a greater decrease than in the case of birch pulp, which may be explained by the improvement in cellulose reactivity.

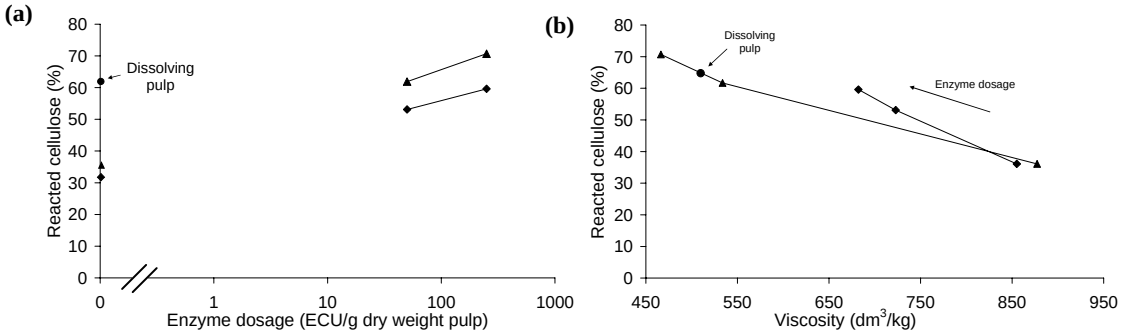

Fig 13. **(a)** Cellulose reactivity of eucalypt pulp as a function of the enzyme dosage and **(b)** viscosity of the pulp after a treatment with: (♦) endoglucanase and (▲) xylanase prior to endoglucanase. Incubation time for endoglucanase: 1 h, and for xylanase: 2 h. Enzyme dosage: 0, 50, and 250 ECU/g dry weight pulp.

3.2.4 Chemical treatment

It is clear that the hemicellulose content must be further decreased for both pulps to accomplish the requirements of a dissolving pulp (<10%). It has been reported previously that, after alkaline extraction, the amount of hemicelluloses is significantly reduced [38]. Therefore, an alkaline extraction step was included after the xylanase treatment. To evaluate the combination of pretreatments, the endoglucanase dosage considered was the one found to give a reactivity value of a commercial dissolving

pulp (250 ECU/g dry weight pulp). In the case of xylanase, 500 EXU/g dry weight pulp was considered since the maximum decrease in hemicelluloses content was reached at this dosage (13%).

Furthermore, both pulps were subjected only to alkaline extraction in order to study and compare the effect of alkaline extraction on the reduction of hemicelluloses and on the cellulose reactivity.

3.2.5 Carbohydrate analysis of the treated pulps

The chemical composition of the pulps after the treatments is presented in *Table 3* for the birch pulp and in *Table 4* for the eucalypt pulp.

Table 3. Chemical composition of birch pulp after different enzymatic and chemical treatments. X: xylanase treatment; AE: alkaline extraction; E: endoglucanase treatment.

Pulp (Birch)	**Glu (%)**	**Man (%)**	**Xyl (%)**	**Ara (%)**	**Gal (%)**
Initial	73.4	<1	25.5	0.4	0.0
X+AE+E	95.2	<1	**3.8**	0.0	0.0
AE	93.9	<1	**5.2**	0.0	0.0

Table 4. Chemical composition of eucalypt pulp after different enzymatic and chemical treatments. X: xylanase treatment; AE: alkaline extraction; E: endoglucanase treatment.

Pulp (Eucalypt)	**Glu (%)**	**Man (%)**	**Xyl (%)**	**Ara (%)**	**Gal (%)**
Initial	78.6	0.0	21.4	0.0	0.0
X+AE+E	97.6	0.0	**2.4**	0.0	0.0
AE	95.9	0.0	**4.1**	0.0	0.0

The above tables show that the alkaline extraction had a marked influence on the decrease of the hemicellulose content for both pulps, which, in the case of the combination of pretreatments, is enhanced by the xylanase. It seems, however, that an alkaline treatment may be sufficient for using kraft-paper grade pulps as dissolving pulps; therefore, cellulose reactivity was also examined.

3.2.6 Reactivity and viscosity

The cellulose reactivity after the combined pretreatments is shown in *Table 5*.

Table 5. Cellulose reactivity, according to Fock, of birch and eucalypt pulp after different enzymatic and chemical treatments. X: xylanase treatment; AE: alkaline extraction; E: endoglucanase treatment.

Pulp	**Reacted cellulose (%)**			
	E	X+E	X+AE+E	AE
Birch	65.6	66.7	66.0	36.8
Eucalypt	59.6	70.7	70.3	24.0

The addition of the alkaline extraction step to the enzymatic pretreatments showed no effect on cellulose reactivity for both pulps. However, the cellulose reactivity was affected negatively when the pulps were subjected only to alkaline extraction.

With only alkaline extraction, the decrease in reactivity may be explained by the hornification effect produced by the removal of hemicelluloses and the further drying of the pulp [49].

On the contrary, for the sequence of pretreatmets, it appears that the addition of the endoglucanase at the end of the pretreatments inhibits the hornification effect caused by the removal of hemicelluloses and, consequently, contributes to the improvement of cellulose reactivity. A hypothesis to this effect may be that the shorter chains (oligosaccharides) resulting from the endoglucanase treatment may locate and occupy the available pores created after the removal of xylan, impeding the formation of new irreversible hydrogen bonds and, therefore, the hornification.

Viscosity is an important parameter in the production of dissolving pulp. Good quality rayon, for instance, requires viscosity values between 200-300 dm^3/kg. As presented in *Table 6*, it was observed that the viscosity significantly increased after alkaline treatment, which was a response of the hornification effect of the pulp after drying. In contrary, for the sequence of pretreatments, viscosity showed a clear decrease when the endoglucanase treatment was added as a final step after the alkaline extraction. It has been suggested that endoglucanases show a high affinity towards the hydrolysis of cellulose II, and this hypothesis may explain the drop in viscosity since, during the alkaline extraction, cellulose II is formed. Nevertheless, this decrease was not reflected in the improvement of cellulose reactivity, which may suggest that there are other factors involved in reactivity.

Table 6. Viscosity of birch and eucalypt pulp after different enzymatic and chemical treatments.
X: xylanase treatment; AE: alkaline extraction; E: endoglucanase treatment.

Pulp	**Viscosity (dm^3/kg)**			
	E	X+E	X+AE+E	AE
Birch	536	466	190	820
Eucalypt	682	474	220	1030

4 Conclusions

It has been demonstrated that enzymatic treatment using a monocomponent endoglucanase can improve the cellulose reactivity, according to the Fock method for the viscose process. The structure of the enzyme (catalytic domain and cellulose-binding domain) plays an important role and must be considered to obtain a maximum effect. A monocomponent endoglucanase with a cellulose-binding domain was shown to notably improve the reactivity compared to a monocomponent endoglucanase without a cellulose-binding domain. Moreover, mechanical treatments may be used as an additional step to further enhance the cellulose reactivity.

The conversion of hardwood paper-grade pulps into dissolving-grade pulps was also demonstrated. The cellulose reactivity values and the hemicellulose content of the pulps obtained after a series of enzymatic and chemical treatments were those of a commercial dissolving pulp. The removal of hemicelluloses was achieved using a combination of a xylanase treatment followed by an alkaline extraction step. Cellulose reactivity, however, was increased by adding an enzymatic treatment with a monocomponent endoglucanase at the end of the series. This last step was also shown to inhibit the hornification effect caused by the removal of hemicelluloses. Furthermore, after the enzymatic treatment, viscosity decreased in all cases.

5 Future work

Other parameters may be involved in the enhancement of the cellulose reactivity. Therefore, in order to understand the mechanism behind the enzymatic treatment, structural analysis should be performed. Also, other techniques to measure cellulose reactivity should be examined for the production of cellulose derivatives and not only for regenerated cellulose. Furthermore, the applicability of these dissolving pulps should be investigated by producing and characterising different cellulose derivatives or regenerated celluloses.

6 Acknowledgements

I thank my supervisor, Associate Professor Monica Ek, for introducing me to this field of science and providing the opportunity to carry out this work in an outstanding research environment.

I wish to express my gratitude to my co-authors, Dr. David Ibarra for his essential comments and inspiring discussions that have been of great value to this study, and Dr. Hiroki Nanko for his input and availability during the first part of this work. Associate Professor Tomas Larsson (STFI Packforsk), Greg DeLozier (Novozymes) and Professor Gunnar Henriksson are thanked for their valuable discussions during the course of this study.

My sincere thanks are to Mona and Inga for all their help whenever it was needed. Also to my colleagues and friends at the Fibre and Polymer department, especially Anders, David, Lotta, Eva-Helena and Helena for being always available for any type of constructive reflection.

I would like to thank my family for their unconditional support and love in spite of the distance, and Moritz for your love, encouragement and understanding.

Vinnova, Bo Rydins and Ångpanneföreningens Forskningsstiftelse are gratefully acknowledged for financial support. Anthony Bristow is thanked for the language revision.

7 References

1. Krässig, H.A., *Cellulose - Structure, Accessibility and Reactivity*. Polymer Monographs, ed. M.B. Huglin. Vol. Vol. 11. 1993, Yverdon: Gordon and Breach Science Publishers.
2. Klemm, D., H.-P. Schmauder, and T. Heinze, *Cellulose.* Biopolymers, 2002. **6**: p. 275-319.
3. Ott, E., et al., *Cellulose and Cellulose Derivatives. Pt. III. 2nd ed*. 1957. 556 pp.
4. Treiber, E.E., *Formation of fibers from cellulose solutions.* Cellul. Chem. Its Appl., 1985: p. 455-79.
5. Sioumis, A.A., *Manufacture of cellulose esters and fibers*. 1991, (Commonwealth Scientific and Industrial Research Organization, Australia). Application: WO
   WO. p. 17 pp.
6. Chanzy, H., M. Paillet, and R. Hagege, *Spinning of cellulose from N-methyl morpholine N-oxide in the presence of additives.* Polymer, 1990. **31**(3): p. 400-5.
7. Fengel, D. and G. Wegener, *Wood: Chemistry, Ultrastructure, Reactions*. 1984. 613 pp.
8. Mantanis, G.I., R.A. Young, and R.M. Rowell, *Swelling of compressed cellulose fiber webs in organic liquids.* Cellulose (London), 1995. **2**(1): p. 1-22.
9. Bochek, A.M., *Effect of Hydrogen Bonding on Cellulose Solubility in Aqueous and Nonaqueous Solvents.* Russian Journal of Applied Chemistry (Translation of Zhurnal Prikladnoi Khimii), 2003. **76**(11): p. 1711-1719.
10. Iovleva, M.M., et al., *Some properties of activated mixtures of cellulose with solid sodium hydroxide.* Khimicheskie Volokna, 1996(5): p. 49-52.
11. Bui, H.M., et al., *Treatment in swelling solutions modifying cellulose fiber reactivity - part 2: accessibility and reactivity.* Macromolecular Symposia, 2008. **262**(Structure and Properties of Cellulose): p. 50-64.
12. Kunze, J. and H.-P. Fink, *Structural changes and activation of cellulose by caustic soda solution with urea.* Macromolecular Symposia, 2005. **223**(Cellulose and Cellulose Derivatives): p. 175-187.
13. Laine, C., et al., *Changes in the fiber wall during refining of bleached pine kraft pulp.* Holzforschung, 2004. **58**(3): p. 233-240.
14. Zhao, H., et al., *Effects of Crystallinity on Dilute Acid Hydrolysis of Cellulose by Cellulose Ball-Milling Study.* Energy & Fuels, 2006. **20**(2): p. 807-811.
15. Isogai, A., F. Onabe, and M. Usuda, *Swelling behavior of cellulose by chemical and mechanical treatments.* Sen'i Gakkaishi, 1992. **48**(9): p. 487-92.
16. Kamaya, Y., *Role of endoglucanase in enzymic modification of bleached kraft pulp.* Journal of Fermentation and Bioengineering, 1996. **82**(6): p. 549-553.
17. Pere, J., et al., *Effects of purified Trichoderma reesei cellulases on the fiber properties of kraft pulp.* Tappi Journal, 1995. **78**(6): p. 71-8.
18. Oksanen, T., et al., *The effect of Trichoderma reesei cellulases and hemicellulases on the paper technical properties of never-dried bleached kraft pulp.* Cellulose (London), 1997. **4**(4): p. 329-339.

19. Bajpai, P., *Application of Enzymes in the Pulp and Paper Industry.* Biotechnology Progress, 1999. **15**(2): p. 147-157.
20. Rahkamo, L., et al., *Modification of hardwood dissolving pulp with purified Trichoderma reesei cellulases.* Cellulose (London), 1996. **3**(3): p. 153-163.
21. Cao, Y. and H. Tan, *Improvement of alkali solubility of cellulose with enzymatic treatment.* Applied Microbiology and Biotechnology, 2006. **70**(2): p. 176-182.
22. Engström, A.-C., M. Ek, and G. Henriksson, *Improved Accessibility and Reactivity of Dissolving Pulp for the Viscose Process: Pretreatment with Monocomponent Endoglucanase.* Biomacromolecules, 2006. **7**(6): p. 2027-2031.
23. Henriksson, G., M. Christiernin, and R. Agnemo, *Monocomponent endoglucanase treatment increases the reactivity of softwood sulphite dissolving pulp.* Journal of Industrial Microbiology & Biotechnology, 2005. **32**(5): p. 211-214.
24. Rahkamo, L., et al., *Effects of cellulases and hemicellulase on the alkaline solubility of dissolving pulps.* Holzforschung, 1998. **52**(6): p. 630-634.
25. Cao, Y. and H. Tan, *Effects of cellulase on the modification of cellulose.* Carbohydrate Research, 2002. **337**(14): p. 1291-1296.
26. Lynd, L.R., et al., *Microbial cellulose utilization: Fundamentals and biotechnology.* Microbiology and Molecular Biology Reviews, 2002. **66**(3): p. 506-577.
27. Eremeeva, T., et al., *Fractionation and molecular characteristics of cellulose during enzymatic hydrolysis.* Cellulose (Dordrecht, Netherlands), 2001. **8**(1): p. 69-79.
28. Mansfield, S.D., C. Mooney, and J.N. Saddler, *Substrate and enzyme characteristics that limit cellulose hydrolysis.* Biotechnology Progress, 1999. **15**(5): p. 804-816.
29. Rabinovich, M.L., M.S. Melnick, and A.V. Bolobova, *The Structure and Mechanism of Action of Cellulolytic Enzymes.* Biochemistry (Moscow, Russian Federation)(Translation of Biokhimiya (Moscow, Russian Federation)), 2002. **67**(8): p. 850-871.
30. Nelson, M.L., et al., *Iodine sorption test. Factors affecting reproducibility and a semimicro adaptation.* Textile Research Journal, 1970. **40**(10): p. 872-80.
31. Hopner, T., G. Jayme, and J.C. Urich, *Determination of water retention (swelling value) of pulps.* Papier (Bingen, Germany), 1955. **9**: p. 476-82.
32. Elg Christoffersson, K., et al., *Reactivity of dissolving pulp: characterisation using chemical properties, NMR spectroscopy and multivariate data analysis.* Cellulose (Dordrecht, Netherlands), 2002. **9**(2): p. 159-170.
33. Fock, W., *A modified method for determining the reactivity of viscose-grade dissolving pulps.* Papier (Bingen, Germany), 1959. **13**: p. 92-5.
34. Kordsachia, O., S. Rosskopf, and R. Patt, *Production of spruce dissolving pulp with the prehydrolysis-alkaline sulfite process (PH-ASA).* Lenzinger Berichte, 2004. **83**: p. 24-34.
35. Sixta, H., et al., *Evaluation of new organosolv dissolving pulps. Part I: Preparation, analytical characterization and viscose processability.* Cellulose (Dordrecht, Netherlands), 2004. **11**(1): p. 73-83.
36. Hillman, D., *Do Dissolving Pulps Really Dissolve?* Paper Asia, 2006: p. 12-18.

37. Christov, L.P. and B.A. Prior, *Xylan removal from dissolving pulp using enzymes of Aureobasidium pullulans.* Biotechnology Letters, 1993. **15**(12): p. 1269-74.
38. Wallis, A.F.A. and R.H. Wearne, *Chemical cellulose from radiata pine kraft pulp.* Appita Journal, 1990. **43**(5): p. 355-7, 366.
39. Janzon, R., J. Puls, and B. Saake, *Upgrading of paper-grade pulps to dissolving pulps by nitren extraction: optimisation of extraction parameters and application to different pulps.* Holzforschung, 2006. **60**(4): p. 347-354.
40. Puls, J., R. Janzon, and B. Saake, *Comparative removal of hemicelluloses from paper pulps using nitren, cuen, NaOH, and KOH.* Lenzinger Berichte, 2006. **86**: p. 63-70.
41. Jackson, L.S., J.A. Heitmann, Jr., and T.W. Joyce, *Production of dissolving pulp from recovered paper using enzymes.* Tappi Journal, 1998. **81**(3): p. 171-178.
42. Theander, O. and E.A. Westerlund, *Studies on dietary fiber. 3. Improved procedures for analysis of dietary fiber.* Journal of Agricultural and Food Chemistry, 1986. **34**(2): p. 330-6.
43. Schulein, M., *Enzymatic properties of cellulases from Humicola insolens.* J Biotechnol FIELD Full Journal Title:Journal of biotechnology, 1997. **57**(1-3): p. 71-81.
44. Linder, M. and T.T. Teeri, *The roles and function of cellulose-binding domains.* Journal of Biotechnology, 1997. **57**(1-3): p. 15-28.
45. Bouchard, J. and M. Douek, *Structural and concentration effects on the diffuse reflectance FTIR spectra of cellulose, lignin and pulp.* Journal of Wood Chemistry and Technology, 1993. **13**(4): p. 481-99.
46. Cao, Y. and H. Tan, *Structural characterization of cellulose with enzymic treatment.* Journal of Molecular Structure, 2004. **705**(1-3): p. 189-193.
47. Gubitz, G.M., et al., *Enzymic removal of hemicellulose from dissolving pulps.* Biotechnology Letters, 1997. **19**(5): p. 491-495.
48. Edgar, C.D., et al., *The synergistic effects of endoglucanase and xylanase in modifying Douglas fir kraft pulp.* ACS Symposium Series, 1998. **687**(Enzyme Applications in Fiber Processing): p. 75-87.
49. Oksanen, T., J. Buchert, and L. Viikari, *The role of hemicelluloses in the hornification of bleached kraft pulps.* Holzforschung, 1997. **51**(4): p. 355-360.

I

The effect of different monocomponent endoglucanases on cellulose accessibility in dissolving pulps

Viviana Köpcke[1], **Hiroki Nanko**[2] and **Monica Ek**[1]
[1]Royal Institute of Technology, KTH, Sweden
[2]Institute of Paper Science and Technology, USA, Current affiliation: MeadWestvaco, USA

KEYWORDS: accessibility, cellulose, reactivity, monocomponent endoglucanase, cellulose-binding domain, mechanical treatment, enzymatic treatment.

SUMMARY: Commercial dissolving pulp from softwood was subjected to enzymatic and mechanical pretreatments to study the effect on cellulose reactivity. The enzyme used was a commercial monocomponent endoglucanase without cellulose-binding domain. With enzymatic treatment only, the reactivity measured according to the Fock method was increased to 90% compared to a value of approximately 70% for the control pulp, but the improvement was less than that found in a previous study using a monocomponent endoglucanase with a cellulose-binding domain (100%). The pulp viscosity was not significantly reduced even at high enzyme dosages, and the yield remained as high as 99%. The enzyme showed a relatively short reaction time of 30 minutes. In an attempt to further increase the cellulose reactivity, the dissolving pulp was subjected to intensive mechanical pretreatment prior to the enzymatic treatment and this mechanical pretreatment raised the reactivity by approximately 6 %-points. At the same time, the pulp viscosity showed a slight decrease and the yield was reduced to approximately 90%. In this case, the reaction time of the enzyme was extremely short. Finally, the effect of the enzyme structure on reactivity was investigated. In this case, the structure of the catalytic module of the enzyme influenced the reactivity more than the presence of a cellulose-binding domain.

INTRODUCTION

At the present time, cellulose fiber production is mainly directed to the pulp and paper industry. Cellulose is also used as raw material for the production of cellulose derivatives and regenerated cellulose, which are used as the primary materials for various products within e.g. the textile, pharmacy and food industries. For instance, rayon, the main commercial product from regenerated cellulose, is mostly produced by the viscose process in which pure cellulose is mercerized using sodium hydroxide and with carbon disulphide converted to cellulose xanthate. The cellulose in solution is then regenerated to the solid state by the addition of sulphuric acid. For environmental reasons, it is desired to reduce the use of carbon disulphide, which is a highly toxic solvent.

In contrast to the production of paper, the production of cellulose derivatives and regenerated cellulose requires a pulp with high cellulose content and, therefore, a low amount of hemicellulose and lignin. These are known as dissolving-grade pulps. Nowadays, these special pulps are produced mainly by two processes: the prehydrolysis kraft and the sulphite process, the latter being used the most.

For the production of high quality products from cellulose derivatives or regenerated cellulose, it is important to achieve a homogeneous substitution of the hydroxyl groups within the cellulose chain by an efficient accessibility to solvents and reagents, and, the improvement of cellulose accessibility and reactivity has therefore become a topic of high interest.

Various treatments have been suggested in order to increase cellulose accessibility in dissolving-grade pulps. Mechanical treatment can, for instance, increase the reactive surface of the fibres and therefore the accessibility of the cellulose by altering the interfibrillar bonds particularly in the primary wall and in the outer lamella of the S1 layer of the cell wall [1]. On the other hand, cellulases, especially monocomponent endoglucanases, have shown a significant ability to enhance cellulose reactivity [2-7].

Cellulases are enzymes that attack the cellulose chain by hydrolyzing the β-1,4-glucosidic bonds. They can be divided into three groups: endoglucanases, cellobiohydrolases and glucosidases. Endoglucanases randomly attack the amorphous sites of the cellulose chain creating shorter chains (oligosaccharides) and therefore new chain ends; cellobiohydrolases act on the cellulose chain by attacking the reducing and non-reducing ends and generating mainly glucose or cellobiose units, and glucosidases act on cellobiose units generating glucose units [8]. It has been suggested that there are three primary parameters affecting the degree of enzymatic hydrolysis: crystallinity, specific surface area and the degree of polymerization of the cellulose [9].

The structure of most cellulases consists of a catalytic domain, which is responsible for the hydrolysis reaction, and a cellulose-binding domain (CBD), which helps the enzyme to bind to the cellulose chain and bring the catalytic domain close to the substrate [8].

Nowadays, there are many industrial applications of cellulases in the textile, detergent and laundry sectors as well as in the paper industry. In detergents, cellulases are used to improve the washing process i.e. the efficient removal of dirt from the object being washed [10]. In the paper industry, endoglucanases are used e.g. to increase the drainage of the pulp [11].

The present study seeks the influence on cellulose reactivity of the enzymatic and mechanical treatment of dissolving pulp. A commercial dissolving pulp of softwood (mixture of Norway spruce and Scots pine) was subjected to enzymatic treatment

using a commercial monocomponent endoglucanase without a cellulose-binding domain. Treatments were performed at different enzyme dosages while the incubation time was kept constant. The pulp was also subjected to an intensive mechanical treatment in combination with the enzymatic treatment. In both cases, the enzyme incubation time was optimized. Results were assessed in terms of reactivity according to a modified Fock method [12], viscosity and yield. The influence of the catalytic domain and the cellulose-binding domain of the enzyme on cellulose reactivity was also investigated.

MATERIALS AND METHODS

Pulp samples

A commercial softwood dissolving pulp from a mixture of Norway spruce (*Picea abies*) and Scots pine (*Pinus sylvestris*) with a lignin content of 0.6% and R18 > 94%, provided by Domsjö Fabriker AB Sweden, was used. The pulp was produced by an acid sulphite process and it was used in a never-dried form.

Enzymes

Three different commercial monocomponent endoglucanases provided by Novozymes, Denmark, were examined. Novozym 476 (N476) is a monocomponent endoglucanase with a cellulose-binding domain. Novozym 613 (N613) is a monocomponent endoglucanase without a cellulose-binding domain, and Novozym 51063 (N51063), also a monocomponent endoglucanase without a cellulose-binding domain. These enzymes are all produced from a genetically modified Aspergillus fungus. The characteristics of the enzymes are summarised in Table 1.

The activities of the enzymes were stated by the manufacturer to be 5000 ECU/g for N476 and N51063, and 2500 ECU/g for N613.

Table 1. Characteristics of the different monocomponent endoglucanases

Name	Enzyme	Cellulose-binding domain
N476	EGV	Yes
N613	EGI	No
N51063	EGV	No

Enzymatic and mechanical treatment

Enzymatic treatments were carried out in a phosphate buffer solution (11 mM NaH_2PO_4 and 9 mM Na_2HPO_4) at pH 7 and 3% pulp consistency. To obtain a homogeneous distribution, the enzyme was first added to the buffer solution and the buffer was then added to the pulp.

All the enzymatic treatments were performed in plastic bags in a water bath at 50ºC. The samples were kneaded every 10-30 minutes depending on the incubation time. To stop the reaction, the enzymes were denaturised by filtration in a Büchner funnel and the sample was then mixed with deionised hot water at 90ºC. The samples were placed in a 90ºC water bath for 30 minutes and finally, filtered and washed with 1000 ml of deionised water. In all cases, a parallel sample of a control pulp was subjected to the same treatment without enzyme.

Different enzyme dosages were chosen depending on the series of experiments under study. For N613 the dosages selected were: 0, 5, 50, 500 and 5000 ECU/g dry weight pulp. The enzyme dosages for N476: 0, 0.05, 0.5, 30 and 50 ECU/g dry weight pulp, were taken from previous studies [4]. In order to investigate the influence of structure of the enzymes, the dosages considered were: 0, 10 and 50 ECU/g dry weight pulp.

The effect of the incubation time during the enzyme treatment was also examined. For these series of experiments, times of: 10, 30, 60, 90 and 120 min. were considered. It must be pointed out that a different cooking batch was used for the study of enzyme incubation time and enzyme structure.

Mechanical treatment was performed using a laboratory grinder (Cerendipitor MKCA6-3, Masuko Sangyo Co., Ltd., Japan). The grinder features two nonporous ceramic grinding discs with an adjustable clearance between the upper and lower discs. While the upper grinding disc is fixed, the lower one is rotated at a high speed. Raw materials, fed into the hopper, are dispersed by centrifugal force into the gap between the upper and lower grinding discs, where they are ground by massive compressive, shearing, and rolling friction forces.

Reactivity of cellulose

Reactivity was measured according to a slightly modified Fock method [4, 12] and expressed in terms of yield of regenerated cellulose. The pulp was dried at 50°C before the analysis.

Viscosity

Viscosity measurements were made according to SCAN-CM 15:99.

Pulp yield

Yield was calculated by gravimetry i.e. the sample was weighed before and after the treatments.

RESULTS AND DISCUSSION

Effect of a monocomponent endoglucanase CBD-free on commercial dissolving pulp

The effect on cellulose reactivity of enzymatic treatment on dissolving pulp using a monocomponent endoglucanase without cellulose-binding domain (N613) was investigated. Previous studies have shown that a monocomponent endoglucanase with cellulose-binding domain (N476) can significantly increase the cellulose reactivity according to the Fock method [4, 5]. These two enzymes were therefore compared in terms of enzyme dosage and reactivity, and the results are illustrated in Fig 1. It was observed that both enzymes improved the cellulose reactivity. This increase in the accessible area may be explained by the fact that the structure of the endoglucanases facilitates the cleavage of the internal bonds of the cellulose due to the rather open active site clefts of the catalytic domain [13]. Cellulose reactivity was enhanced by about 20 %-points using N613, although, it was noticed that even at high enzyme dosages (5000 ECU/g dry weight pulp) the reactivity could not reach values higher than 90%. In contrast, in comparison with N613, it was demonstrated that the N476, even at low dosages (30 ECU/g dry weight pulp) showed reactivity values of 100 % [2].

The lack of a cellulose-binding domain (CBD) in N613 may be one factor involved in this lower enhancement in reactivity since the presence of a CBD facilitates the binding of the enzyme to the substrate [8, 13, 14]. The difference in the structure of the catalytic domain may also explain this effect since N613 is an endoglucanase I (EG I), and N476 is an endoglucanase V (EG V). Endoglucanases act preferably on amorphous rather than crystalline cellulose [15] and it has been shown that EG V presents a higher catalytic rate towards low ordered cellulose than EG I [16].

The lower enhancement in reactivity is also supported by the lower decrease in pulp viscosity for the pulp treated with N613, as illustrated in Fig 2, where viscosity was affected but not to a great extent. In contrary, the pulp treated with the N476 presented a more marked decrease in viscosity as expected.

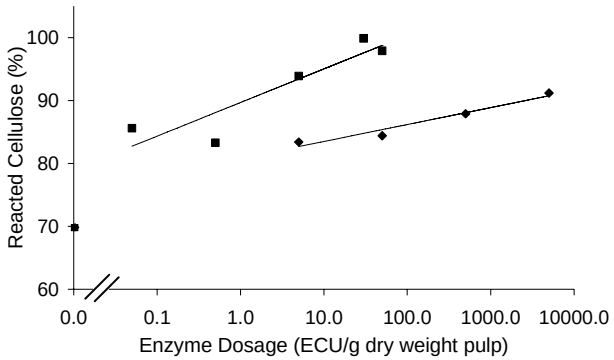

Fig 1. Reactivity according to Fock method after enzymatic treatment with a monocomponent endoglucanase with CBD (N476) (■) and CBD-free (N613) (♦). Incubation time: 1 h. The values for N476 are taken from a previous study [4].

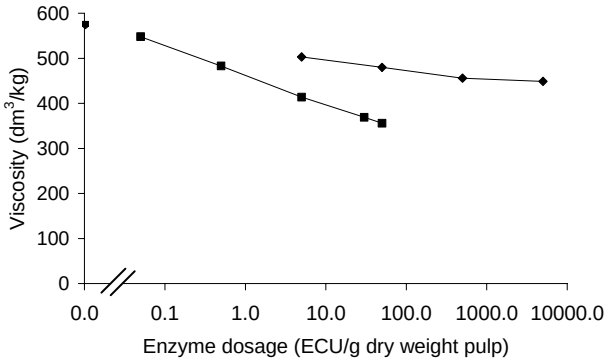

Fig 2. Pulp viscosity after enzymatic treatment with N476 (■) and N613 (♦). Incubation time: 1 h. The values for the N476 were taken from a previous study [4].

Optimisation of the enzymatic treatment time

The reaction time of the N613 on dissolving pulp was examined. An enzyme dosage of 30 ECU/g dry weight pulp was selected in order to compare the behaviour of this enzyme with the monocomponent endoglucanase with cellulose-binding domain (N476) for which the results were presented in a previous report [4]. Fig 3 shows that the enzyme reacted fairly rapidly since after 30 minutes, the reactivity was constant. Similar behaviour was reported for N476, with a reaction time of 10 min [4].

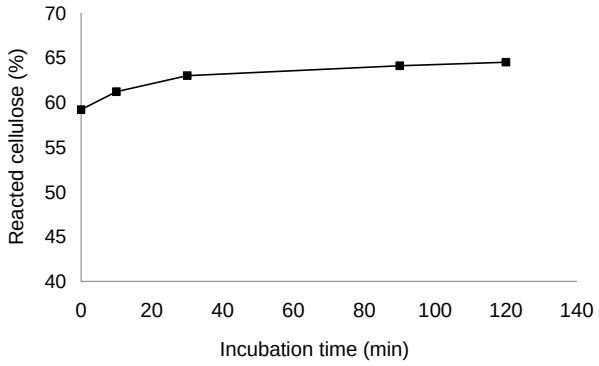

Fig 3. Reactivity of enzyme-treated dissolving pulp, according to Fock, as a function of incubation time. Enzyme dosage: 30 ECU/g dry weight pulp.

Mechanical treatment

The accessibility to cellulose and the reactive surface can be increased by grinding the pulp [1]. Therefore, the effect of mechanical treatment prior to enzymatic treatment using N613 was also investigated. The effect of the mechanical treatment was examined by light microscopy and it was observed that the fibres had become highly fibrillated as shown in Fig 4.

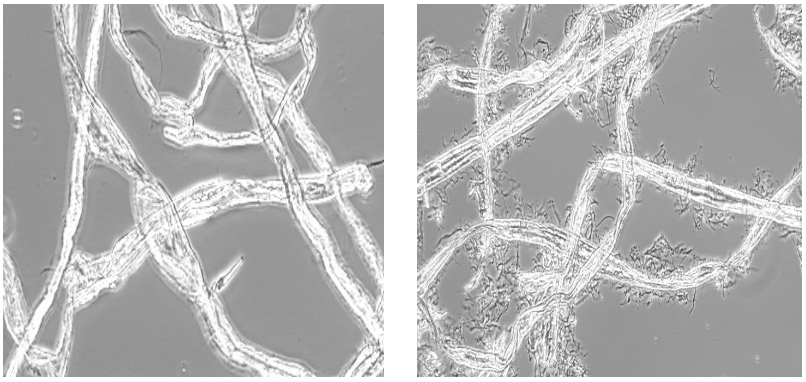

Fig 4. Commercial dissolving pulp: untreated (left) and, after being subjected to an intensive mechanical treatment (right).

The effect on the reactivity of different enzyme dosages is shown in Fig 5. After an intensive mechanical treatment prior to enzymatic treatment, the reactivity of the dissolving pulp was greater than that of the pulp which was subjected only to enzymatic treatment. The improvement in reactivity was given mainly by the mechanical treatment and it was about 10 %-points with respect to the control pulp (Fig.5). This improvement can be explained by the fibrillation during mechanical treatment, which mainly affects the outer layers of the cell wall (primary wall and outer lamella of the S1), and leads to a larger available surface area [1]. The effect of mechanical treatment may also facilitate the entry of enzyme into the fibres, resulting in a better performance and contributing to the improvement in the cellulose reactivity.

It was noticed that when the pulp was subjected only to mechanical treatment, the reactivity was the same as that of a pulp treated with an enzyme dosage of 50 ECU/g dry weight pulp. Nevertheless, in despite of the further improvement in reactivity, it was evident that mechanical treatment prior to enzymatic treatment with N613, even a high enzyme dosages, could not raise the reactivity to values of 100 % as it had been reached with N476 [4].

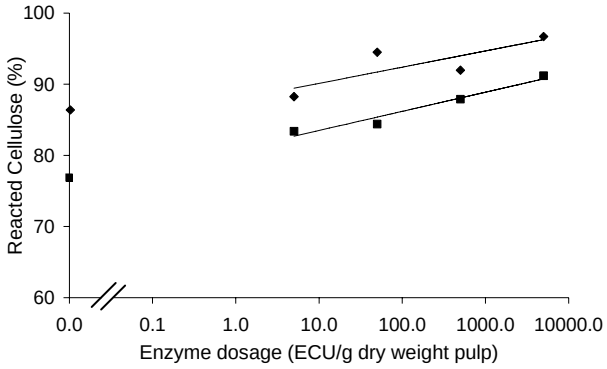

Fig 5. Reactivity according to the Fock method of a commercial dissolving pulp subjected to (■) enzymatic treatment with N613 and (♦) intensive mechanical treatment followed by enzymatic treatment with N613. Enzyme dosages: 5, 50, 500, 5000 ECU/g dry pulp. Incubation time: 1 h.

The effect on the pulp viscosity of mechanical treatment is shown in Fig 6, where it is evident that mechanical treatment prior to the enzymatic treatment had only a slight effect. At low enzyme dosages, the viscosity was the same for both samples, which implied that the mechanical treatment did not affect the viscosity, but at higher enzyme dosages the effect of the mechanical treatment increased and enhanced the performance of the enzyme.

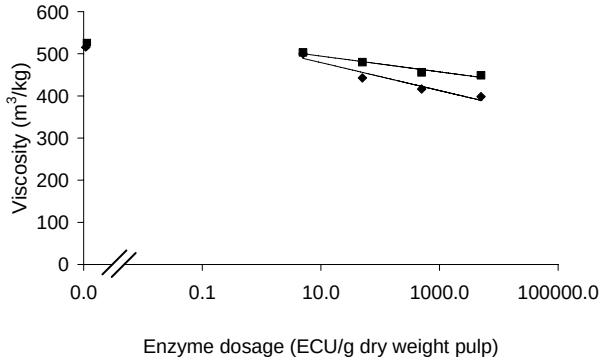

Fig 6. Comparison in viscosity of a commercial dissolving pulp subjected to (■) enzymatic treatment with a N613 only and (♦) intensive mechanical treatment followed by enzymatic treatment with a N613. Enzyme dosage: 5, 50, 500, 5000 ECU/g dry pulp. Incubation time: 1 h.

Effect of the enzyme incubation time

The effect of the mechanical treatment on the enzyme reaction rate was also assessed and it is illustrated in Fig 7. There was an immediate response of the enzyme towards cellulose compared to the slower response (30 minutes) of the enzyme alone, and there was no variation in reactivity when the incubation time was increased. The addition of mechanical treatment prior to the enzymatic treatment thus increased the reaction rate of the enzyme significantly by increasing the available area due to the fibrillation effect.

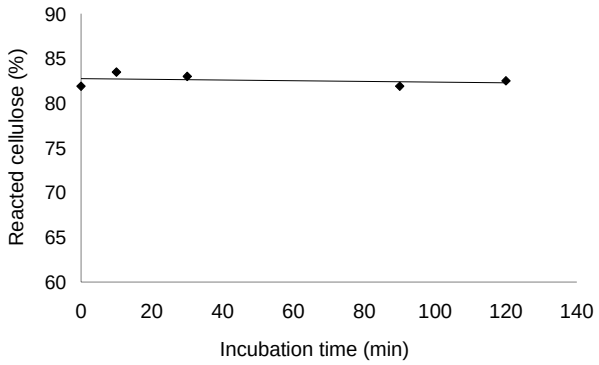

Fig 7. Reactivity of mechanically and enzymatically treated dissolving pulp, according to Fock, as a function of the incubation time. Enzyme dosage: 30 ECU/g dry weight pulp.

Effect on the pulp yield

The pulp yield with and without the addition of mechanical treatment to the enzymatic treatment was determined. In the case of the enzyme-treated pulp, the pulp yield was around 99%, but when mechanical treatment was included, the yield decreased to 90%. There may be two explanations for this. Firstly, the mechanical treatment leads to a fibrillation of the cell wall and therefore an increase in the production of fines, which may be lost after filtration of the pulp with a loss in yield. Secondly, in order to subject the pulp to the intensive mechanical treatment, the pulp had to be passed through the laboratory-scale grinder 11 times, and this also led to a loss in yield.

Effect of reactivity due to enzyme structure

The structure of the enzyme plays an important role for its reactivity with cellulose. In the present work, the monocomponent endoglucanase without cellulose-binding domain (N613) enhanced the cellulose reactivity but not as much as the monocomponent endoglucanase with cellulose-binding domain (N476) even when the pulp was subjected to mechanical treatment prior to the enzymatic treatment. Thus, the presence of a cellulose-binding domain in the enzyme structure appears to have a strong influence on the reactivity.

Both enzymes in study, N613 and N476, are monocomponent endoglucanases, and apart from the difference regards the cellulose-binding domain, they present different catalytic domains: the N476 belongs to the EGV and the N613 to the EGI group. Therefore, in order to investigate which parameter had the greatest influence on the performance of the enzyme towards cellulose reactivity, the study of a third enzyme was necessary.

The new enzyme chosen, N51063, belongs to the EGV group and has the same catalytic domain as the N476 but lacks a cellulose-binding domain.

The three enzymes: N613, N476 and N51063, were compared with regard to reactivity, according to the Fock method, at different enzyme dosages of: 0, 10 and 50 ECU/g dry weight pulp. The results are presented in Fig. 8.

It was observed, that not only the presence of a cellulose-binding domain, but also the structure of the catalytic domain enhanced reactivity. The N51063 showed approximately the same increase in reactivity as the N476 when the enzyme dosage was increased, which suggests that the cellulose-binding domain enhances the reactivity when a moncomponent endoglucanase CBD-free is used.

In this case, however, it is observed that N613 showed the lowest values of reactivity at different enzyme dosages which suggests that the structure of the catalytic domain of the N613 had a major impact on the performance towards cellulose reactivity of the dissolving pulp than the presence of a cellulose-binding domain.

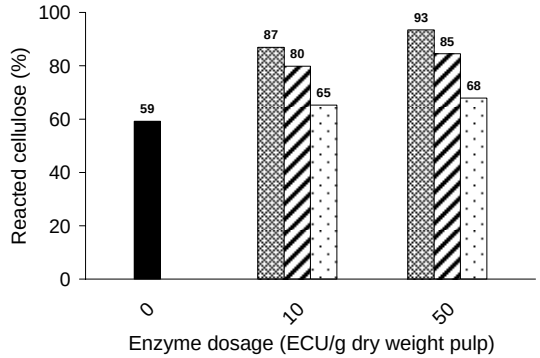

Fig 8. The cellulose reactivity, according to Fock for N476 (□), N51063 (▨) and N613 (⊡) at two enzyme dosages and a control pulp without enzymatic treatment (■). Incubation time: 1 h.

To summarize, it can be seen that in all cases the cellulose reactivity was enhanced to some extent, suggesting that the increase in reactivity depended mainly on the structure of the enzyme (catalytic domain). Moreover, it has been suggested in previous studies that the presence of a CBD has little effect on the activity of the

enzyme towards amorphous cellulose but a high effect towards crystalline cellulose [14].

These results may not be generally applicable to all types of dissolving pulps. All monocomponent endoglucanases do not show the same effect towards cellulose reactivity, and the performance of these enzymes may differ for dissolving pulps based on hardwoods, or for dissolving pulps produced by a prehydrolysis kraft process.

CONCLUSIONS

- A monocomponent endoglucanase without a cellulose-binding domain enhanced the cellulose reactivity, according to the Fock method, of dissolving pulp, but it was not as efficient as a monocomponent endoglucanase containing a cellulose-binding domain.

- A mechanical treatment prior to enzymatic treatment resulted in a further increase in cellulose reactivity of the dissolving pulp being the main contributor to this enhancement.

- Mechanical treatment enhanced the accessibility to the enzyme, which was reflected in a decrease in pulp viscosity. The yield, on the other hand, was markedly decreased by this treatment.

- The enzyme reacted rapidly towards dissolving pulp. This effect accentuated after a mechanical treatment.

- The structure of the enzyme plays an important role for the cellulose reactivity, and in this particular study it was noticed that the structure of the catalytic domain had a greater impact on the enhancement of cellulose reactivity of the dissolving pulp than the presence of a cellulose-binding domain.

ACKNOWLEDGEMENTS

VINNOVA/Tekes is acknowledged for financial support for the project “New Cellulose Derivatives from Wood for High Value Products” (NewCell). Domsjö Fabriker AB is thanked for the supplying the dissolving pulp and Novozyme, Denmark is thanked for providing the enzymes. Greg Delozier of Novozyme, and Dr. David Ibarra of KTH are especially thanked for valuable discussions.

REFERENCES

1. Krässig, H.A., *Cellulose - Structure, Accessibility and Reactivity*. Polymer Monographs, ed. M.B. Huglin. Vol. Vol. 11. 1993, Yverdon: Gordon and Breach Science Publishers.
2. Rahkamo, L., et al., *Modification of hardwood dissolving pulp with purified Trichoderma reesei cellulases.* Cellulose (London), 1996. **3**(3): p. 153-163.
3. Cao, Y. and H. Tan, *Improvement of alkali solubility of cellulose with enzymatic treatment.* Applied Microbiology and Biotechnology, 2006. **70**(2): p. 176-182.
4. Engström, A.-C., M. Ek, and G. Henriksson, *Improved Accessibility and Reactivity of Dissolving Pulp for the Viscose Process: Pretreatment with Monocomponent Endoglucanase.* Biomacromolecules, 2006. **7**(6): p. 2027-2031.
5. Henriksson, G., M. Christiernin, and R. Agnemo, *Monocomponent endoglucanase treatment increases the reactivity of softwood sulphite dissolving pulp.* Journal of Industrial Microbiology & Biotechnology, 2005. **32**(5): p. 211-214.
6. Rahkamo, L., et al., *Effects of cellulases and hemicellulase on the alkaline solubility of dissolving pulps.* Holzforschung, 1998. **52**(6): p. 630-634.
7. Cao, Y. and H. Tan, *Effects of cellulase on the modification of cellulose.* Carbohydrate Research, 2002. **337**(14): p. 1291-1296.
8. Lynd, L.R., et al., *Microbial cellulose utilization: Fundamentals and biotechnology.* Microbiology and Molecular Biology Reviews, 2002. **66**(3): p. 506-577.
9. Eremeeva, T., et al., *Fractionation and molecular characteristics of cellulose during enzymatic hydrolysis.* Cellulose (Dordrecht, Netherlands), 2001. **8**(1): p. 69-79.
10. Gibson, K. and L. Hansen, *Detergent composition and enzymatically improving detergency in washing fabrics*. 2004, (Novozymes A/S, Den.). Application: WO
WO. p. 71 pp.
11. Leclerc, M., *Use of cellulose preparation for treatment of wastepaper, recycled paperboard, and pulps*. 1992, (Novo Nordisk A/S, Den.). Application: WO
WO. p. 17 pp.
12. Fock, W., *A modified method for determining the reactivity of viscose-grade dissolving pulps.* Papier (Bingen, Germany), 1959. **13**: p. 92-5.
13. Teeri, T.T., *Fiber modification in nature: modes of action of cellulolytic enzymes.* International Conference on Biotechnology in the Pulp and Paper Industry, 7th, Vancouver, B. C., June 16-19, 1998, 1998. **A**: p. A171-A174.
14. Linder, M. and T.T. Teeri, *The roles and function of cellulose-binding domains.* Journal of Biotechnology, 1997. **57**(1-3): p. 15-28.
15. Rabinovich, M.L., M.S. Melnick, and A.V. Bolobova, *The Structure and Mechanism of Action of Cellulolytic Enzymes.* Biochemistry (Moscow, Russian Federation)(Translation of Biokhimiya (Moscow, Russian Federation)), 2002. **67**(8): p. 850-871.
16. Schulein, M., *Enzymatic properties of cellulases from Humicola insolens.* J Biotechnol FIELD Full Journal Title:Journal of biotechnology, 1997. **57**(1-3): p. 71-81.

II

Increasing accessibility and reactivity of paper grade pulp by enzymatic treatment for use as dissolving pulp

Viviana Köpcke, David Ibarra and **Monica Ek,** Royal Institute of Technology, KTH, Sweden

KEYWORDS: Hardwood, Kraft pulp, Dissolving pulp, Reactivity, Xylanase, Monocomponent endoglucanase, Alkaline extraction.

SUMMARY: In this study, the feasibility of using different kraft pulps as dissolving pulps for the viscose process was investigated. Two different bleached hardwood kraft pulps from eucalypt *(Eucalyptus globulus)* and birch (*Betula pendula*) were subjected to several enzymatic and chemical pretreatments in order to improve the accessibility and reactivity of the pulps and to reduce the hemicellulose content. Enzymatic treatments were carried out using a commercial monocomponent endoglucanase and a commercial xylanase. Chemical treatment consisted of an alkaline extraction. The effects of these pretreatments on reactivity and viscosity were assayed. In both pulps, the endoglucanase enhanced the cellulose reactivity and reduced the viscosity. The sequential combination of xylanase and endoglucanase enhanced the positive effect of endoglucanase treatment alone for eucalypt but showed no major effect for birch. The addition of an alkaline extraction step after the xylanase followed by endoglucanase treatment as a final step significantly reduced the hemicellulose content to 2-4% while the reactivity reached the value of a commercial dissolving pulp (65-70%). The viscosity, on the other hand, showed a considerably decrease. FTIR spectra of enzymatic-treated pulps showed a decrease in the hydrogen bonding energy compared to that of the reference pulp.

ADDRESSES OF THE AUTHORS: Viviana Köpcke (vivianah@kth.se), **David Ibarra** (ibarra@kth.se) and **Monica Ek** (monicaek@kth.se): Royal Institute of Technology, School of Chemical Sciences and Engineering, Division of Wood Chemistry and Pulp Technology, Teknikringen 56-58, SE-100 44 Stockholm, Sweden
Corresponding author: Monica Ek

INTRODUCTION

Cellulose constitutes an important polymer in nature, and is the raw material for several industrial products. Most cellulose is used in the pulp and paper industry, but a substantial part is needed in the production of cellulose-based products that have important applications in the pharmaceutical, textile, food and paint industries. The manufacture of these products requires the dissolution of cellulose but, due to the strong fibrilar structure of cellulose, its dissolution is restricted to a cheap and common solvent such as carbon disulphide which is a highly toxic solvent used in the viscose process (Treiber 1985). Alternative solvents have been tested. NMMO for instance, is an environmentally acceptable but expensive solvent that has been used for the production of Lyocell (Chanzy et al. 1990).

Most manufacturer of regenerated cellulose, e.g. rayon, use the viscose process in which cellulose prepared from either wood pulp or less commonly cotton linters, is treated with sodium hydroxide and carbon disulphide to make it soluble as cellulose xanthate. This step is followed by the regeneration of fibres from the solution to a solid state in an acid solution.

Raw material with a high cellulose content is required for viscose manufacture and, for this purpose, special dissolving-grade pulps are produced. In contrast to paper-grade pulp, dissolving pulp must contain a high content of cellulose and low contents of hemicelluloses, residual lignin, extractives and minerals. Besides, high brightness and preferably a uniform molecular weight distribution are desired. Dissolving-grade pulp is today mainly manufactured by acid sulphite and pre-hydrolysis kraft processes. In addition, organosolv processes, especially Formacell which uses acetic acid as solvent, have been suggested as an alternative pulping method (Sixta et al. 2004).

In recent years, several studies have focused on the viability of converting paper-grade kraft pulp into dissolving-grade pulp, concentrating mainly on the removal of hemicelluloses. In contrast to paper-grade pulps, hemicelluloses are undesirable in the viscose process since they can affect viscose filterability and xanthation of the cellulose, as well as the strength of the end product (Christov, Prior 1993). Different pretreatments have been presented in order to achieve the removal of hemicelluloses, including alkaline extraction (Wallis, Wearne 1990), and nitren and cuen extraction (Janzon et al. 2006; Puls et al. 2006). In the same way, the potential of enzymes to reduce the hemicellulose content has motivated an interest in their application in the manufacture of paper and dissolving pulps. Xylanase treatment (Bajpai, Bajpai 2001) and xylanase in combination with alkaline extraction (Jackson et al. 1998) have shown positive results.

In the production of regenerated cellulose or cellulose derivatives, it is also an advantage to have a homogeneous substitution along the cellulose chain. This is not a simple task since the strong hydrogen bonds give the chains a compact structure (Fengel, Wegener 1984). It is, therefore, important to increase the accessibility of the cellulose to solvents and reactants in order to improve the homogeneity and quality of regenerated cellulosic products, and at the same time to reduce the demand for carbon disulphide. Various pretreatments have been assessed to increase the reactivity of the cellulose (Krässig 1993), including the use of enzymes such as monocomponent cellulases and cellulase mixtures on dissolving-grade pulps, which have shown a positive effect (Rahkamo et al. 1996; Henriksson et al. 2005; Engström et al. 2006).

In this study, the main focus has been to investigate the influence of various enzymatic and chemical pretreatments on several paper-grade kraft pulps, in order to assess the feasibility of using them as raw material for viscose. For this purpose, not only the decrease in hemicellulose content, but also the accessibility and reactivity of the pulp have been investigated. Two different commercial hardwood pulps have been examined since the use of hardwood instead of softwood has increased in recent years due to lower costs and better availability (Sixta et al. 2004). Commercial dried ECF-bleached kraft pulps from eucalypt

(*E.globulus*) and birch (*B.pendula*) were subjected to different enzymatic and chemical pretreatments using a commercial fungal monocomponent endoglucanase, a commercial xylanase and an alkaline extraction step. The optimization of these pretreatments in terms of enzyme dosage, incubation time and a possible combination of them was investigated. The reactivity of the pulp was determined by a modified Fock method (Fock 1959), a micro-scale process similar to viscose manufacture. Fourier-transform infrared (FTIR) spectroscopy was used to confirm the effect of the enzymatic treatment on the cellulose structure.

MATERIALS AND METHODS

Pulp samples

Commercial dried ECF-bleached kraft pulp from eucalypt (*E.globulus*) (kappa number 0.94, ISO brightness 91% and viscosity 863 dm^3/kg) provided by Ence (Spain) and dried ECF-bleached kraft pulp from birch (*B.pendula*) (kappa number 0.77, ISO brightness 90% and viscosity 771 dm^3/kg), provided by Metsä Botnia (Finland) were used. Commercial dried TCF-bleached sulphite dissolving pulp from eucalypt (*E.globulus*) (ISO brightness 91% and viscosity 530 dm^3/kg) with α-cellulose 92% and hemicellulose content 2.4%, provided by Sniace (Spain), was used as a reference. Prior to the treatments, the dried sheets were soaked in water for 24 h and disintegrated in an L&W equipment at 1.5% consistency and 30 000 revolutions.

Enzymes

A monocomponent endoglucanase preparation (Novozyme 476) and a xylanase preparation (Pulpzyme HC), both provided by Novozymes Denmark, were used. Novozyme 476 is produced from a genetically modified *Aspergillus* species. The cellulolytic activity was determined by the manufacturer and expressed in Endo-cellulose Units (ECU) per unit mass of material as 5000 ECU/g. Pulpzyme HC is produced from a genetically modified *Bacillus* species. The xylanase activity was determined by the manufacturer and expressed in Endo-xylanase Units (EXU) per units of material as 1000 EXU/g.

Enzymatic and chemical treatments

Enzymatic treatments were performed at 3% pulp consistency at pH 7 using a phosphate buffer solution (11 mM NaH_2PO_4 and 9 mM Na_2HPO_4). To achieve a homogeneous distribution, the enzyme was added to the buffer and then to the pulp. The enzymatic incubation was performed in plastic bags in a water bath at 50ºC with the monocomponent endoglucanase, and at 60ºC with the xylanase. The pulps were kneaded every 30 minutes. After treatment, the enzyme was denaturised with hot water, by filtration, using a Büchner funnel. The pulp was then, mixed with deionised water at 90ºC, put in a 90ºC water bath for 30 minutes and subsequently filtered and washed with 1000 ml of deionised water.

The enzyme dosage and incubation time for the monocomponent endoglucanase were optimised for both pulps. Different enzyme dosages were tested: 0, 5, 30, 50, 250 and 500 ECU/g dry weight pulp, keeping the incubation time constant at 1 h, and different

incubation times of 0, 10, 30, 45 and 60 min, keeping the enzyme dosage constant at 250 ECU/g dry weight pulp.

In the same way, in order to optimize the removal of xylan by enzymatic treatment, different dosages of xylanase: 10, 80 and 500 EXU/g dry weight pulp, were tested with an incubation time of 2 h. In all cases, a control pulp was treated in parallel under identical conditions without enzyme.

Chemical treatment consisted of alkaline extraction with 9% NaOH solution at room temperature for 1 h. The consistency of the pulp was 4%.

Reactivity measurements

The reactivities of all the pulps were assessed according to a slightly modified Fock method (Fock 1959; Henriksson et al. 2005). Reactivity was expressed as the yield of regenerated cellulose. Before the analysis, the pulps were dried at 50°C.

Determination of viscosity

Viscosity was determined according to SCAN-CM 15:99.

Carbohydrate analysis

The carbohydrate composition of the pulps was analyzed after acid hydrolysis, reduction with sodium borohydride and subsequent acetylation (Theander, Westerlund 1986). The samples were examined by gas chromatography (GC) using a Hewlett-Packard HP-6890 chromatograph.

Fourier transform-infrared spectroscopy (FTIR)

FTIR spectra were recorded on a Perkin-Elmer 2000 FTIR spectrometer. The wave number range scanned was 4000-700 cm^{-1}.

RESULTS AND DISCUSSION

Effect of monocomponent endoglucanase on birch and eucalypt pulps

Reactivity and viscosity

The positive effect of the monocomponent endoglucanase of a dissolving pulp, in terms of reactivity according to Fock, has been presented in earlier studies (Henriksson et al. 2005; Engström et al. 2006). In this study, a similar effect was observed with this enzyme on the kraft pulps.

Both pulps – birch and eucalypt – treated with a monocomponent endoglucanase showed a significant increase in reactivity compared to the respective references (*Fig 1)*. Previous

studies on dissolving-grade pulps have shown that the reactivity increases drastically (90-100%) using relatively small amounts of enzyme (Henriksson et al. 2005; Engström et al. 2006). In the case of both kraft pulps, the reactivity was enhanced by the enzyme reaching values close to that of a commercial reference dissolving pulp (60-65%) at an enzyme dosage of 250 ECU/g. At higher dosages, a very small increase in reactivity was observed.

The enhancement in reactivity may be explained by the hypothesis that the enzyme attacks the less ordered regions between and on the surface of the fibrils, leading to a greater accessibility to solvents and therefore enhancing the reactivity of the pulp (Engström et al. 2006). The limited rise in reactivity can possibly be attributed to the hemicellulose, mainly glucuroxylan in hardwood, located on the surface of the pulp fibres hindering the penetration of the endoglucanase into the fibres. The size of the enzyme may also limit the attack within the fibrils with stretched bonds reducing the accessibility and leaving only the surface areas available.

Another important parameter in the production of dissolving pulp is the viscosity. Good quality rayon, for instance, requires viscosity values between 200-300 dm^3/kg. When birch and eucalypt pulps were subjected to enzymatic treatment, both pulps showed a notable decrease in viscosity when the enzyme dosage was increased, as illustrated in *Fig 2*. The decrease in viscosity is attributed to the cleavage of the polymer chain by the endoglucanase. The same tendency has been demonstrated for hardwood and softwood dissolving pulps (Cao, Tan 2002; Engström et al. 2006) as well as for softwood kraft pulps (Pere et al. 1995). It can be observed also that the viscosity of the birch pulp is affected by the enzyme more than the eucalypt pulp. The differences in viscosity may be explained by differences in pulp structure e.g. the eucalypt may contain more crystalline regions. Both pulps show the same trend, i.e. in both cases the viscosity exhibited a marked decrease when the pulp was treated up to 50 ECU/g, although at higher dosages the viscosity decreased disproportionately less.

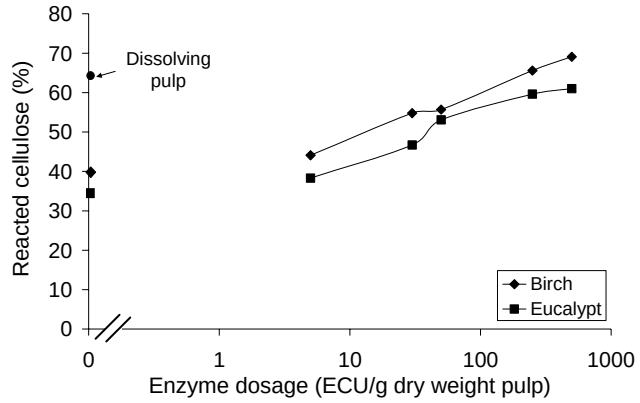

Fig 1. Reactivity of enzyme-treated birch and eucalypt pulps, according to Fock, as a function of enzyme dosage (represented in a logarithmic scale). Incubation time: 1 h.

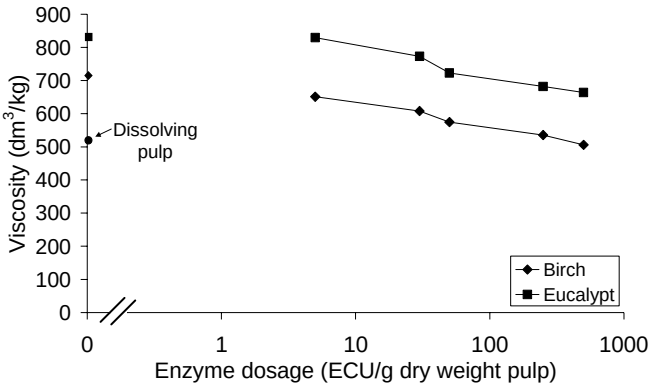

Fig 2. Viscosity of the enzyme-treated birch and eucalypt pulps as a function of enzyme dosage. Incubation time: 1 h.

Structural analysis by FTIR

The effect of the enzymatic treatment on the structure of the cellulose was studied by FTIR analysis (*Fig 3* and *Fig 4).* Both spectra showed typical cellulose peaks around 1000-1200 cm^{-1} (Bouchard, Douek 1993). The bands at 3300 cm^{-1} correspond to OH vibrations of intermolecular and intramolecular hydrogen bonds of cellulose fibrils. After enzymatic treatment, the OH vibration bands of both pulps were narrower than that of the control pulp. This suggests that the hydrogen bond energy has been changed and the hydrogen bonds broken by the endoglucanase treatment. The same effect has been demonstrated on dissolving pulps treated with other endoglucanases (Cao, Tan 2004).

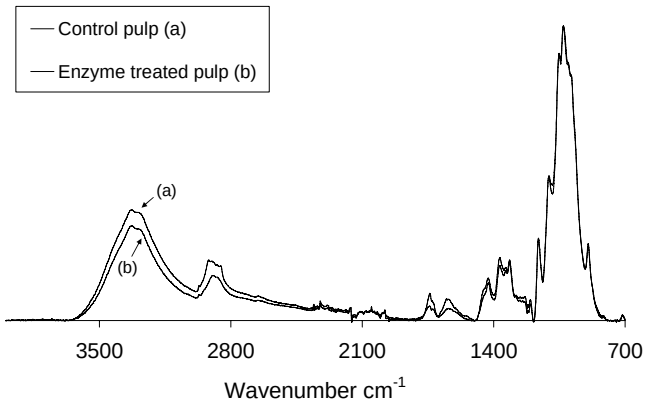

Fig 3. FTIR spectra for birch pulp treated with 250 ECU/g dry weight pulp endoglucanase and the control pulp. Incubation time: 1 h.

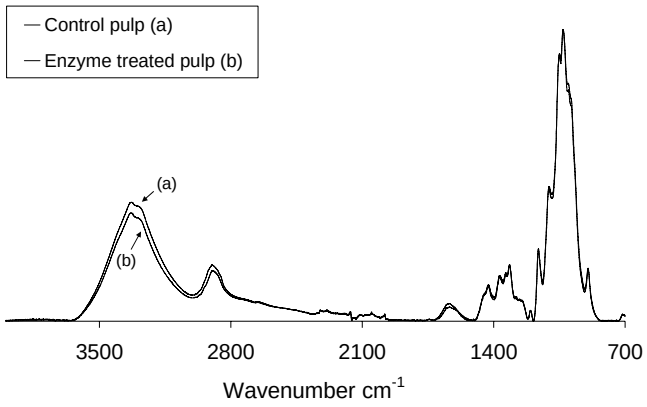

Fig 4. FTIR spectra for eucalypt pulp treated with 250 ECU/ g dry weight pulp endoglucanase and the control pulp. Incubation time: 1 h.

Optimisation of enzymatic treatment time

The reactivities of both pulps were studied as a function of enzyme incubation time. *Fig 5* shows that both pulps presented the same tendency and that the main increase in reactivity occurred during the first 15 minutes. In birch the reactivity reached a maximum value, after 30 minutes; but in eucalypt the reactivity continued to increase slightly after 30 minutes. This behaviour of the eucalypt confirmed the lower reactivity to enzymatic treatment compared with the birch pulp. Eucalypt pulp may contain more aggregated fibrils and this might be a cause of the slower reaction with the enzyme.

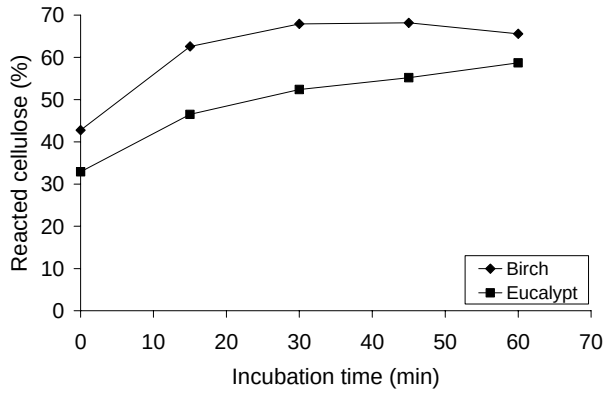

Fig 5. Reactivity of enzyme-treated birch and eucalypt kraft pulps, according to Fock, as a function of incubation time. Enzyme dosage: 250 ECU/g dry weight pulp.

Effect of xylanase treatment on birch and eucalypt kraft pulps

Xylan content and carbohydrate analysis

An important parameter in viscose manufacture is the hemicellulose content. By definition, dissolving pulp has a high cellulose content (90-99%) and low hemicellulose content (1-10%), since the latter is considered to be an undesirable impurity. As shown in *Table 1*, both

pulps contained a high amount of hemicelluloses, and in order to reduce the amount of xylan, they were subjected to enzymatic treatment using different dosages of xylanase (*Fig 6*).

Table 1. Carbohydrate analysis of birch and eucalypt kraft pulps

Pulp	**Glu (%)**	**Man (%)**	**Xyl (%)**	**Ara (%)**	**Gal (%)**
Birch	73.4	<1	25.5	0.4	0.0
Eucalypt	78.6	0.0	21.4	0.0	0.0

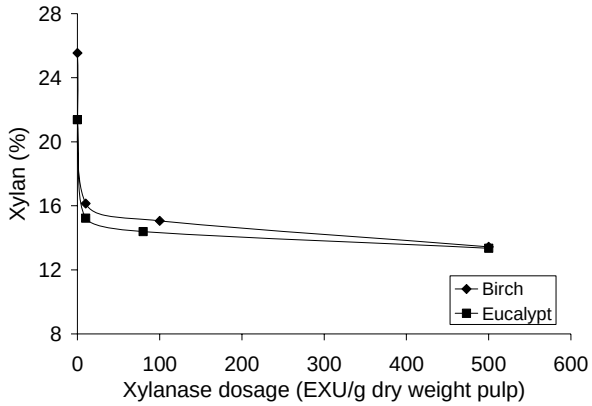

Fig 6. Xylan content of birch and eucalypt kraft pulps as a function of xylanase dosage after 2 h incubation time.

The amount of xylan in both types of pulp decreased considerably at a low dosage. At enzyme dosages higher than 500 EXU/g, the amount of xylan could not be further decreased. In birch and eucalypt, 47% and 37% respectively of the initial xylan was solubilised by the enzymatic treatment. The remaining xylan content in both pulps was around 13%. To explain this behaviour, Gübitz et al. (1997) have suggested that, in contrast to unbleached pulp, the hemicelluloses in bleached pulp may have been chemically modified altering their sensitivity to xylanase attack, or that the accessible hemicelluloses were dissolved during bleaching. The hemicellulose- lignin linkage may also restrict the efficiency of the enzyme (Christov, Prior 1993), and, the size of the enzyme may limit its ability to reach and penetrate the inner regions within the fibres.

For neither of the pulps was the reactivity affected after the hemicellulose removal by xylanase treatment (data not shown). One reason for this may be that the removal of hemicellulose enhanced hornification of the pulp, and to some extent affected the cellulose reactivity.

Effect of a sequential treatment using xylanase prior to the monocomponent endoglucanase on birch and eucalypt kraft pulps

Reactivity and viscosity

After investigating the optimal xylanase dosage (500 EXU/g dry weight pulp) and the amount of xylan removed, a sequential enzymatic treatment with xylanase and endoglucanase was investigated in terms of reactivity. The results are presented in *Fig 7* and *Fig 8*.

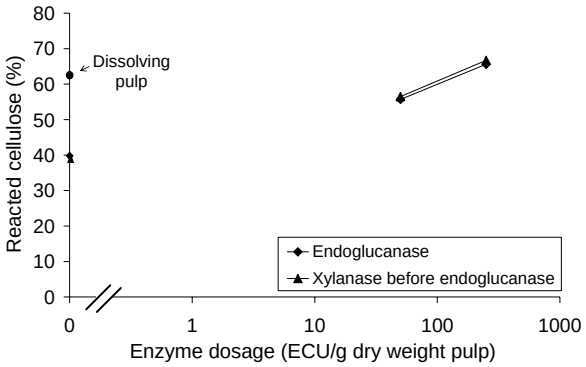

Fig 7. Reactivity, according to Fock, of sequential enzymatic treatment for birch kraft pulp at different endoglucanase dosages (represented in a logarithmic scale). Endoglucanase dosage: 0, 50 and 250 ECU/g dry weight pulp at 1h incubation time. Xylanase dosage: 500 EXU/g dry weight pulp at 2 h incubation time.

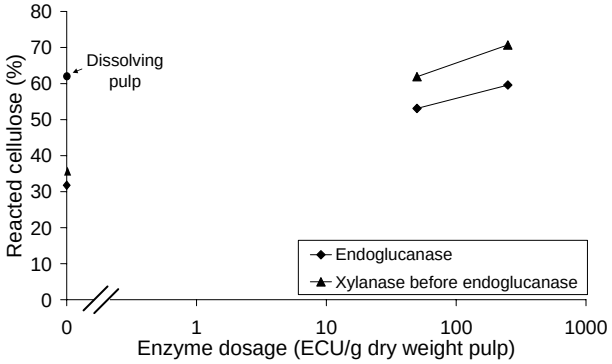

Fig 8. Reactivity, according to Fock, of sequential enzymatic treatment for eucalypt kraft pulp at different endoglucanase dosage (represented in a logarithmic scale). Endoglucanase dosage: 0, 50 and 250 ECU/g dry weight pulp at 1 h incubation time. Xylanase dosage: 500 EXU/g dry weight pulp at 2 h incubation time.

It is observed that the addition of xylanase prior to endoglucanase treatment had only a very slight influence in the case of birch pulp (*Fig 7*) but a significant effect in the case of eucalypt pulp (*Fig 8*). At 250 ECU/g the reactivity was equivalent to or even higher (71%) in case of eucalypt, than that of commercial dissolving pulp. A synergistic effect of xylanase and endoglucanase together has been reported previously (Edgar et al. 1998) and, the present results show that the combination of enzymatic treatments positively affected the reactivity of the eucalypt pulp but had no noticeable effect in case of birch pulp. These results may be explained by the hypothesis that the removal of xylan, by the xylanase, enhances the accessibility to endoglucanase by increasing the pore size within the fibres. In the case of

birch pulp, the enhancement in reactivity by the endoglucanase seems not to be dependant on the removal of xylan.

Fig 9 and *Fig 10* show the relationship between reacted cellulose and viscosity for the two pulps after the sequential treatments. The trend is the same in both cases, but eucalypt pulp showed a greater decrease in viscosity when xylanase was added before the endoglucanase. This effect might be explained by the greater increase in reactivity shown by this pulp. In contrast, birch pulp showed a slight decrease in viscosity after the xylanase treatment even though the reactivity was not affected by the addition of this enzyme prior to endoglucanase (*Fig 6)*. This suggests that there are other parameters, besides viscosity, that affect cellulose reactivity.

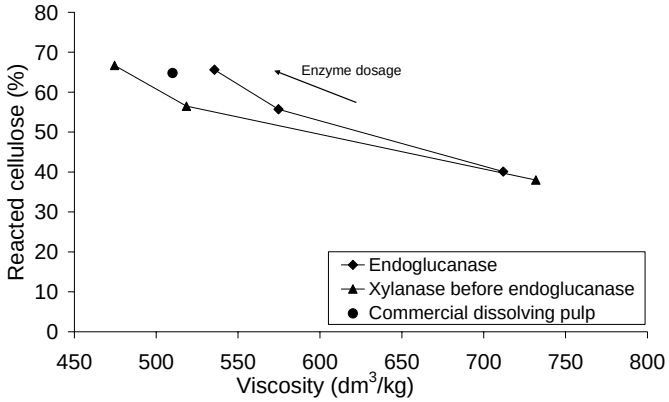

Fig 9. Relation between reacted cellulose and viscosity for birch pulp. Endoglucanase dosage: 0, 50 and 250 ECU/ g dry weight pulp. Xylanase dosage: 500 EXU/ g dry weight pulp.

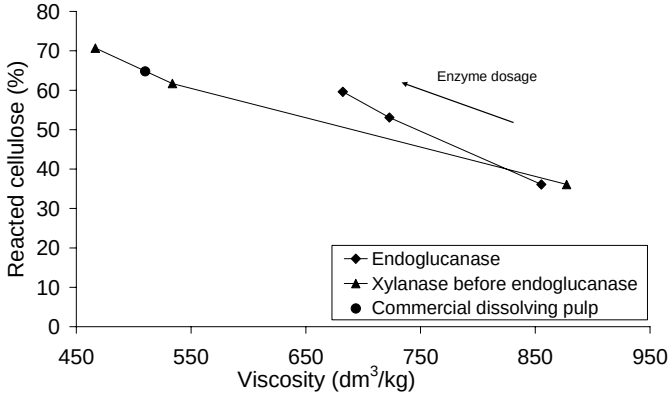

Fig 10. Relation between reacted cellulose and viscosity for eucalypt pulp. Endoglucanase dosage: 0, 50 and 250 ECU/ g dry weight pulp. Xylanase dosage: 500 EXU/ g dry weight pulp.

Effect of alkaline extraction on birch and eucalypt kraft pulps

Reduction in the hemicellulose content by alkaline extraction (carbohydrate analysis)

In order to achieve the hemicellulose content required for a dissolving pulp, the pulps were subjected to an alkaline extraction. Pulps treated with alkali alone and as an intermediate between the xylanase and the endoglucanase treatment were investigated in terms of chemical composition. The results are presented in *Table 2* and *Table 3*.

Table 2. Carbohydrate analysis of (A) birch pulp treated with xylanase (500 EXU/g dry weight pulp) followed by alkaline extraction and treatment with endoglucanase (250 ECU/g dry weight pulp) and (B) birch pulp after alkaline extraction.

Pulp (Birch)	**Glu (%)**	**Man (%)**	**Xyl (%)**	**Ara (%)**	**Gal (%)**
A	95.2	<1	**3.8**	0.0	0.0
B	93.9	<1	**5.2**	0.0	0.0

Table 3. Carbohydrate analysis of (A) eucalypt pulp treated with xylanase (500 EXU/g dry weight pulp) followed by alkaline extraction and treatment with endoglucanase (250 ECU/g dry weight pulp) and (B) eucalypt pulp after alkaline extraction.

Pulp (Eucalypt)	**Glu (%)**	**Man (%)**	**Xyl (%)**	**Ara (%)**	**Gal (%)**
A	97.6	0.0	**2.4**	0.0	0.0
B	95.9	0.0	**4.1**	0.0	0.0

The two pulps showed similar results. As expected and reported earlier (Rahkamo et al. 1998), approximately 85% of the initial xylan was solubilised by the alkaline extraction. The fact that eucalypt pulp showed a lower xylose content than birch after the pretreatments, is explained by the smaller amount of xylose in the raw material. It also appeared that, in order to use kraft-paper grade pulps as dissolving-grade pulps, a pretreatment with alkali should be sufficient. However, since reactivity also plays an important role for dissolving pulp quality; the reactivity was also investigated.

Reactivity and viscosity

The reactivity of the different pretreatments was investigated in order to examine the effect of using alkaline extraction solely or in combination with xylanase and endoglucanase treatments. The results are presented in *Table 4* and *Table 5*.

Table 4. Reactivity, according to Fock, of birch kraft pulp after a combination of pretreatments involving endoglucanase dosage 250 ECU/g dry weight pulp at 1h incubation time, and xylanase dosage 500 EXU/g dry weight content at 2 h incubation time.

Pretreatment	Reacted cellulose (%)
Endoglucanase	65.6
Xylanase prior to endoglucanase	66.7
Xylanase followed by alkaline extraction prior to endoglucanase	66.0
Alkaline extraction	36.8

Table 5. Reactivity, according to Fock, of eucalypt kraft pulp after a combination of pretreatments involving endoglucanase dosage 250 ECU/g dry weight pulp at 1h incubation time, and xylanase dosage 500 EXU/g dry weight content at 2 h incubation time.

Pretreatment	Reacted cellulose (%)
Endoglucanase	59.6
Xylanase prior to endoglucanase	70.7
Xylanase followed by alkaline extraction prior to endoglucanase	70.3
Alkaline extraction	24.0

After alkaline extraction, the great reduction in the hemicelluloses content affected the reactivity of the pulp negatively. This can be explained as being an effect of hornification during drying (Oksanen et al. 1997). It can also be noticed that the addition of endoglucanase as a final step inhibited the hornification effect caused by the alkaline treatment and contributed to the enhancement in reactivity reaching reactivity values of equivalent to those of a commercial hardwood dissolving pulp (64%).

The contribution of endoglucanase to the reactivity may be explained by the creation of short cellulose chains as a result of the cleavage of the polymer after endoglucanase attack. These chains may locate and therefore occupy the available pores within the fibres formed after xylan removal, thus impeding the formation of new hydrogen bonds and hindering the hornification effect.

The effect of an alkaline extraction step on the viscosity was also studied. *Table 6* shows that viscosity increased when the pulp was treated under alkaline conditions. This result is explained by a hornification of the pulp and the decrease in reactivity obtained.

A significantly drop in the degree of polymerization was also observed when endoglucanase was added as a final step. This could be due to the tendency for the endoglucanase to hydrolyse cellulose II (Atalla 1979) since, during alkaline extraction, cellulose II was formed.

Nevertheless, as illustrated in *Table 4* and *Table 5*, the reactivity seems not to be affected. Since the reactivity does not only increase with a decrease in viscosity, some other mechanism apparently affects the activation of the pulp achieved by the endoglucanase.

Table 6. Viscosity of birch and eucalypt after different pretreatments

Pulp	**Pretreatment**	**Viscosity (dm^3/kg)**
Birch	Xylanase followed by alkaline extraction prior to endoglucanase	190
	Alkaline extraction	820
Eucalypt	Xylanase followed by alkaline extraction prior to endoglucanase	220
	Alkaline extraction	1030

CONCLUSIONS

The feasibility of using birch and eucalypt kraft pulp as a dissolving pulp has been demonstrated. It has been shown that a monocomponent endoglucanase improves the reactivity of both pulps. The best combination of pretreatments, in terms of reactivity, viscosity and hemicellulose content, was found to be a xylanase treatment followed by alkaline extraction and finally an endoglucanase treatment. This combination had a positive effect on reactivity, which reached a level comparable with that of a commercial dissolving pulp.

It has been demonstrated that the monocomponent endoglucanase gives the greatest improvement in cellulose reactivity, impeding the hornification effect associated with the alkaline treatment. No correlation was however found between the increase in reactivity and the decrease in viscosity, which suggests that other factors are involved.

The introduction of an alkaline extraction after xylanase treatment greatly enhances the reduction of xylan content to 2.5% for eucalypt and 3.8 % for birch pulp.

The viscosity decreased as a result of all the pretreatments. The decrease was greatest when an alkaline extraction was included in the pretreatment sequence.

ACKNOWLEDGEMENTS

Dr. D.I. acknowledges a grant from Jacob Wallenberg Research Foundation. Associate Professor Tomas Larsson is acknowledged for valuable discussion. Ence (Spain), Sniace (Spain) and Metsä Botnia (Finland) are acknowledged for providing the pulp samples. Novozymes (Denmark) is acknowledged for supplying the enzymes.

REFERENCES

Atalla, R.H. (1979): Conformational effects of the hydrolysis of cellulose. Advances in Chemistry Series 181(Hydrolysis Cellul.: Mech. Enzym. Acid Catal.), 55-69.

Bajpai, P. and Bajpai, P.K. (2001): Development of a process for the production of dissolving kraft pulp using xylanase enzyme. Appita Journal 54(4), 381-384.

Bouchard, J. and Douek, M. (1993): Structural and concentration effects on the diffuse reflectance FTIR spectra of cellulose, lignin and pulp. Journal of Wood Chemistry and Technology 13(4), 481-99.

Cao, Y. and Tan, H. (2002): Effects of cellulase on the modification of cellulose. Carbohydrate Research 337(14), 1291-1296.

Cao, Y. and Tan, H. (2004): Structural characterization of cellulose with enzymic treatment. Journal of Molecular Structure 705(1-3), 189-193.

Chanzy, H., Paillet, M. and Hagege, R. (1990): Spinning of cellulose from N-methyl morpholine N-oxide in the presence of additives. Polymer 31(3), 400-5.

Christov, L.P. and Prior, B.A. (1993): Xylan removal from dissolving pulp using enzymes of Aureobasidium pullulans. Biotechnology Letters 15(12), 1269-74.

Edgar, C.D., Mansfield, S.D., Gubitz, G.M. and Saddler, J.N. (1998): The synergistic effects of endoglucanase and xylanase in modifying Douglas fir kraft pulp. ACS Symposium Series 687(Enzyme Applications in Fiber Processing), 75-87.

Engström, A.-C., Ek, M. and Henriksson, G. (2006): Improved Accessibility and Reactivity of Dissolving Pulp for the Viscose Process: Pretreatment with Monocomponent Endoglucanase. Biomacromolecules 7(6), 2027-2031.

Fengel, D. and Wegener, G. (1984): Wood: Chemistry, Ultrastructure, Reactions.

Fock, W. (1959): A modified method for determining the reactivity of viscose-grade dissolving pulps. Papier (Bingen, Germany) 13, 92-5.

Gubitz, G.M., Lischnig, T., Stebbing, D. and Saddler, J.N. (1997): Enzymic removal of hemicellulose from dissolving pulps. Biotechnology Letters 19(5), 491-495.

Henriksson, G., Christiernin, M. and Agnemo, R. (2005): Monocomponent endoglucanase treatment increases the reactivity of softwood sulphite dissolving pulp. Journal of Industrial Microbiology & Biotechnology 32(5), 211-214.

Jackson, L.S., Heitmann, J.A., Jr. and Joyce, T.W. (1998): Production of dissolving pulp from recovered paper using enzymes. Tappi Journal 81(3), 171-178.

Janzon, R., Puls, J. and Saake, B. (2006): Upgrading of paper-grade pulps to dissolving pulps by nitren extraction: optimisation of extraction parameters and application to different pulps. Holzforschung 60(4), 347-354.

Krässig, H.A. (1993): Cellulose - Structure, Accessibility and Reactivity. Yverdon, Gordon and Breach Science Publishers.

Oksanen, T., Buchert, J. and Viikari, L. (1997): The role of hemicelluloses in the hornification of bleached kraft pulps. Holzforschung 51(4), 355-360.

Pere, J., Siika-aho, M., Buchert, J. and Viikari, L. (1995): Effects of purified Trichoderma reesei cellulases on the fiber properties of kraft pulp. Tappi Journal 78(6), 71-8.

Puls, J., Janzon, R. and Saake, B. (2006): Comparative removal of hemicelluloses from paper pulps using nitren, cuen, NaOH, and KOH. Lenzinger Berichte 86, 63-70.

Rahkamo, L., Siika-aho, M., Vehvilainen, M., Dolk, M., Viikari, L., Nousiainen, P. and Buchert, J. (1996): Modification of hardwood dissolving pulp with purified Trichoderma reesei cellulases. Cellulose (London) 3(3), 153-163.

Rahkamo, L., Viikari, L., Buchert, J., Paakkari, T. and Suortti, T. (1998): Enzymic and alkaline treatments of hardwood dissolving pulp. Cellulose (London) 5(2), 79-88.

Sixta, H., Harms, H., Dapia, S., Parajo, J.C., Puls, J., Saake, B., Fink, H.P. and Roeder, T. (2004): Evaluation of new organosolv dissolving pulps. Part I: Preparation, analytical characterization and viscose processability. Cellulose (Dordrecht, Netherlands) 11(1), 73-83.
Sixta, H., Promberger, A., Koch, G., Gradinger, C. and Messner, K. (2004): Influence of beech wood quality on bisulfite dissolving pulp manufacture. Part 1: influence of log storage on pulping and bleaching. Holzforschung 58(1), 14-21.
Theander, O. and Westerlund, E.A. (1986): Studies on dietary fiber. 3. Improved procedures for analysis of dietary fiber. Journal of Agricultural and Food Chemistry 34(2), 330-6.
Treiber, E.E. (1985): Formation of fibers from cellulose solutions. Cellul. Chem. Its Appl., 455-79.
Wallis, A.F.A. and Wearne, R.H. (1990): Chemical cellulose from radiata pine kraft pulp. Appita Journal 43(5), 355-7, 366.